



**EFSA Panel on Food Contact Material, Enzymes, Flavourings and Processing Aids (CEF); Scientific Opinion on Flavouring Group Evaluation 51, Revision 1: Consideration of alicyclic ketones and secondary alcohols and related esters evaluated by the JECFA (59th meeting) structurally related to alicyclic ketones secondary alcohols and related esters in FGE.09Rev3 (2011)**

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## SCIENTIFIC OPINION

### Scientific Opinion on Flavouring Group Evaluation 51, Revision 1 (FGE.51Rev1):

#### Consideration of alicyclic ketones and secondary alcohols and related esters evaluated by the JECFA (59<sup>th</sup> meeting) structurally related to alicyclic ketones secondary alcohols and related esters in FGE.09Rev3 (2011)<sup>1</sup>

#### EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)<sup>2, 3</sup>

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#### ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to consider evaluations of flavouring substances assessed since 2000 by the Joint FAO/WHO Expert Committee on Food Additives (the JECFA), and to decide whether further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000. The present consideration concerns a group of 20 alicyclic ketones and secondary alcohols and related esters evaluated by JECFA (59<sup>th</sup> meeting) in 2002. This revision is made due to inclusion of seven additional substances cleared for genotoxicity concern compared to the previous version. The substances were evaluated through a stepwise approach that integrates information on structure-activity relationships, intake from current uses, toxicological threshold of concern, and available data on metabolism and toxicity. The Panel agrees with the application of the Procedure as performed by the JECFA for all 20 substances considered in this FGE and agrees with the JECFA conclusion, “No safety concern at estimated levels of intake as flavouring substances” based on the MSDI approach. Besides the safety assessment of these flavouring substances, the specifications for the materials of commerce have also been considered and for all 20 substances, the information is adequate.

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1 On request from the European Commission, Question No (EFSA-Q-2011-01040; EFSA-Q-2011-01041; EFSA-Q-2011-01042; EFSA-Q-2011-01043; EFSA-Q-2011-01044; EFSA-Q-2011-01045; EFSA-Q-2011-01046), adopted on 22 March 2012.

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**KEY WORDS**

Alicyclic ketones, JECFA 59th meeting, alicyclic secondary alcohols, FGE.09.

## SUMMARY

The Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) was asked to give scientific advice to the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel was requested to consider the Joint FAO/WHO Expert Committee on Food Additives (the JECFA) evaluations of flavouring substances assessed since 2000, and to decide whether no further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000. These flavouring substances are listed in the Register, which was adopted by Commission Decision 1999/217/EC and its consecutive amendments.

This revision of FGE.51 is made due to the consideration of the seven the alpha,beta-unsaturated substances [FL-no: 07.034, 07.035, 07.098, 07.126, 07.129, 07.172 and 09.930] compared to the previous version of FGE.51. Furthermore, EU production volume on one substance [FL-no: 09.230] and data on stereoisomerism for four substances [FL-no: 02.209, 07.045, 07.095 and 07.257] have been provided since the publication of FGE.51.

The JECFA has evaluated a group of 25 flavouring substances consisting of alicyclic ketones, secondary alcohols and related esters at its 59<sup>th</sup> meeting. Two of the JECFA-evaluated substances are not in the Register (4-methyl cyclohexanone (JECFA no: 1104) and (E)-2-(2-octenyl) cyclopentanone (JECFA no: 1116)) and ten of the substances are alpha,beta-unsaturated ketones or precursors for such, which is recognized as a structural alert for genotoxicity. Seven of these 10 alpha,beta-unsaturated substances [FL-no: 07.034, 07.035, 07.098, 07.126, 07.129, 07.172 and 09.930] have been evaluated with respect to their genotoxic potential in FGE.211 (EFSA, 2011e) or in FGE.212Rev1 (EFSA, 2011f), and the Panel concluded that the data available ruled out the concern for genotoxicity and accordingly these seven substances can be evaluated through the Procedure.

The present consideration therefore concerns 20 alicyclic ketones, secondary alcohols and related esters evaluated by the JECFA at its 59<sup>th</sup> meeting and will be considered in relation to the European Food Safety Authority (EFSA) evaluation of 17 secondary alicyclic saturated and unsaturated alcohols, ketones and esters containing secondary alicyclic alcohols evaluated in the Flavouring Group Evaluation 09, Revision 3 (FGE.09Rev3).

The Panel agrees with the application of the Procedure as performed by the JECFA for the 20 substances considered in this FGE.

For all substances evaluated through the Procedure use levels are needed to calculate the mTAMDI in order to identify those flavouring substances that need more refined exposure assessment and to finalise the evaluation.

In order to determine whether the conclusion for the JECFA-evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity tests are available for all 20 substances evaluated in this FGE.51Rev1.

For all 20 evaluated alicyclic ketones, secondary alcohols and related esters [FL-no: 02.209 07.034, 07.035, 07.045, 07.095, 07.098, 07.126, 07.129, 07.148, 07.149, 07.172, 07.179, 07.180, 07.257, 09.027, 09.140, 09.160, 09.230, 09.464 and 09.930], the Panel agrees with the JECFA conclusion, "No safety concern at estimated levels of intake as flavouring substance" based on the MSDI approach.

## TABLE OF CONTENTS

|  |    |
|--|----|
| Abstract .....   | 1  |
| Summary .....  | 3  |
| Table of contents .....  | 4  |
| Background .....   | 5  |
| Terms of Reference .....   | 5  |
| Assessment .....   | 5  |
| History of the Evaluation of the substances in the present FGE .....   | 7  |
| 1. Presentation of the Substances in the JECFA Flavouring Group .....  | 8  |
| 1.1. Description.....  | 8  |
| 1.1.1. JECFA Status.....   | 8  |
| 1.1.2. EFSA Considerations .....   | 8  |
| 1.2. Isomers .....   | 8  |
| 1.2.1. Status .....  | 8  |
| 1.2.2. EFSA Considerations .....   | 8  |
| 1.3. Specifications.....   | 8  |
| 1.3.1. JECFA Status.....   | 8  |
| 1.3.2. EFSA Considerations .....   | 8  |
| 2. Intake Estimations .....  | 9  |
| 2.1. JECFA Status.....   | 9  |
| 2.2. EFSA Considerations.....  | 9  |
| 3. Genotoxicity Data.....  | 9  |
| 3.1. Genotoxicity Studies – Text Taken from the JECFA (JECFA, 2003a) .....   | 9  |
| 3.2. Genotoxicity Studies - Text Taken from EFSA FGE.09Rev3 (EFSA, 2011x).....   | 11 |
| 3.3. Genotoxicity Studies - Text Taken from EFSA FGE.211 (EFSA, 2011e).....  | 12 |
| 3.4. Genotoxicity Studies - Text Taken from EFSA FGE.212 (EFSA, 2009ai) and<br>FGE.212Rev1 (EFSA, 2011f).....  | 14 |
| 3.5. EFSA Considerations.....  | 16 |
| 4. Application of the Procedure .....  | 16 |
| 4.1. Application of the Procedure to 20 Alicyclic Ketones, Secondary Alcohols or Related<br>Esters Evaluated by the JECFA (JECFA, 2003a):.....   | 16 |
| 4.2. Application of the Procedure to 17 Secondary Alicyclic Saturated and Unsaturated<br>Alcohols, Ketones and Esters Containing Secondary Alicyclic Alcohols by EFSA in FGE.09Rev3<br>(EFSA, 2011x):..... | 17 |
| 4.3. EFSA Considerations.....  | 17 |
| 5. Conclusions .....   | 17 |

## BACKGROUND

Regulation (EC) No 2232/96 of the European Parliament and the Council (EC, 1996a) lays down a Procedure for the establishment of a list of flavouring substances, the use of which will be authorised to the exclusion of all other substances in the EU. In application of that Regulation, a Register of flavouring substances used in or on foodstuffs in the Member States was adopted by Commission Decision 1999/217/EC (EC, 1999a), as last amended by Commission Decision 2009/163/EC (EC, 2009a). Each flavouring substance is attributed a FLAVIS-number (FL-number) and all substances are divided into 34 chemical groups. Substances within a group should have some metabolic and biological behaviour in common.

Substances which are listed in the Register are to be evaluated according to the evaluation programme laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000a), which is broadly based on the Opinion of the Scientific Committee on Food (SCF, 1999a).

Commission Regulation (EC) No 1565/2000 lays down that substances that are contained in the Register and will be classified in the future by the Joint FAO/WHO Expert Committee on Food Additives (the JECFA) so as to present no safety concern at current levels of intake will be considered by the European Food Safety Authority (EFSA), who may then decide that no further evaluation is necessary.

In the period 2000 - 2008, during its 55<sup>th</sup>, 57<sup>th</sup>, 59<sup>th</sup>, 61<sup>st</sup>, 63<sup>rd</sup>, 65<sup>th</sup>, 68<sup>th</sup> and 69<sup>th</sup> meetings, the JECFA evaluated about 1000 substances, which are in the EU Register.

## TERMS OF REFERENCE

The European Food Safety Authority (EFSA) is requested to consider the JECFA evaluations of flavouring substances assessed since 2000, and to decide whether no further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000a). These flavouring substances are listed in the Register which was adopted by Commission Decision 1999/217 EC (EC, 1999a) and its consecutive amendments.

The evaluation programme was finalised at the end of 2009.

After the finalisation of the evaluation programme, in their letters of the 7<sup>th</sup> May 2010 and 3<sup>rd</sup> June 2010, the Commission requested EFSA to carry out re-evaluation of the flavouring substances, tetramethyl ethylcyclohexenone [FL-no: 07.035], 3-methylcyclohex-2-en-1-one [FL-no: 07.098], 3,5,5-trimethylcyclohex-2-en-1-one (isophorone) [FL-no: 07.126], 3-methyl-5-propylcyclohex-2-en-1-one [FL-no: 07.129], 4-isopropylcyclohex-2-en-1-one [FL-no: 07.172], 2-hexylidenecyclopentan-1-one [FL-no: 07.034] and 1(7),8-p-menthadien-2-yl acetate (mixture of (E) and (Z) isomers) [FL-no: 09.930] based on additionally submitted data on genotoxicity, and depending on the outcome, to proceed to the evaluation of these flavouring substances through the Procedure, also according to Commission Regulation (EC) No 1565/2000 (EC, 2000a).

## ASSESSMENT

The approach used by EFSA for safety evaluation of flavouring substances is referred to in Commission Regulation (EC) No 1565/2000 (EC, 2000a), hereafter named the “EFSA Procedure”. This Procedure is based on the Opinion of the Scientific Committee on Food (SCF, 1999a), which has been derived from the evaluation procedure developed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1995; JECFA, 1996a; JECFA, 1997a; JECFA, 1999b), hereafter named the “JECFA Procedure”. The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) compares the JECFA evaluation of structurally related substances with the result of a corresponding EFSA evaluation, focussing on specifications, intake estimations and toxicity data, especially genotoxicity data. The evaluations by EFSA will conclude whether the flavouring

substances are of no safety concern at their estimated levels of intake, whether additional data are required or whether certain substances should not be evaluated through the EFSA Procedure.

The following issues are of special importance.

#### *Intake*

In its evaluation, the Panel as a default uses the “Maximised Survey-derived Daily Intake” (MSDI) approach to estimate the *per capita* intakes of the flavouring substances in Europe.

In its evaluation, the JECFA includes intake estimates based on the MSDI approach derived from both European and USA production figures. The highest of the two MSDI figures is used in the evaluation by the JECFA. It is noted that in several cases, only the MSDI figures from the USA were available, meaning that certain flavouring substances have been evaluated by the JECFA only on the basis of these figures. For Register substances for which this is the case the Panel will need EU production figures in order to finalise the evaluation.

When the Panel examined the information provided by the European Flavour Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by the Industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about the data on use and use levels provided and the intake estimates obtained by the MSDI approach. It is noted that the JECFA, at its 65<sup>th</sup> meeting considered “how to improve the identification and assessment of flavouring agents, for which the MSDI estimates may be substantially lower than the dietary exposures that would be estimated from the anticipated average use levels in foods” (JECFA, 2006c).

In the absence of more accurate information that would enable the Panel to make a more realistic estimate of the intakes of the flavouring substances, the Panel has decided also to perform an estimate of the daily intakes per person using a “modified Theoretical Added Maximum Daily Intake” (mTAMDI) approach based on the normal use levels reported by Industry.

As information on use levels for the flavouring substances has not been requested by the JECFA or has not otherwise been provided to the Panel, it is not possible to estimate the daily intakes using the mTAMDI approach for the substances evaluated by the JECFA. The Panel will need information on use levels in order to finalise the evaluation.

#### *Threshold of 1.5 Microgram/Person/Day (Step B5) Used by the JECFA*

The JECFA uses the threshold of concern of 1.5 microgram/person/day as part of the evaluation Procedure:

“The Committee noted that this value was based on a risk analysis of known carcinogens which involved several conservative assumptions. The use of this value was supported by additional information on developmental toxicity, neurotoxicity and immunotoxicity. In the judgement of the Committee, flavouring substances for which insufficient data are available for them to be evaluated using earlier steps in the Procedure, but for which the intake would not exceed 1.5 microgram per person per day would not be expected to present a safety concern. The Committee recommended that the Procedure for the Safety Evaluation of Flavouring Agents used at the forty-sixth meeting be amended to include the last step on the right-hand side of the original procedure (“Do the condition of use result in an intake greater than 1.5 microgram per day?”)” (JECFA, 1999b).

In line with the Opinion expressed by the Scientific Committee on Food (SCF, 1999), the Panel does not make use of this threshold of 1.5 microgram per person per day.



### *Genotoxicity*

As reflected in the Opinion of SCF (SCF, 1999a), the Panel has in its evaluation focussed on a possible genotoxic potential of the flavouring substances or of structurally related substances. Generally, substances for which the Panel has concluded that there is an indication of genotoxic potential *in vitro*, will not be evaluated using the EFSA Procedure until further genotoxicity data are provided. Substances for which a genotoxic potential *in vivo* has been concluded, will not be evaluated through the Procedure.

### *Specifications*

Regarding specifications, the evaluation by the Panel could lead to a different opinion than that of JECFA, since the Panel requests information on e.g. isomerism.

### *Structural Relationship*

In the consideration of the JECFA evaluated substances, the Panel will examine the structural relationship and metabolism features of the substances within the flavouring group and compare this with the corresponding FGE.

## **HISTORY OF THE EVALUATION OF THE SUBSTANCES IN THE PRESENT FGE**

At its 59<sup>th</sup> meeting the JECFA evaluated a group of 25 flavouring substances consisting of alicyclic ketones, secondary alcohols and related esters. Two substances were not in the Register, and 10 are alpha,beta-unsaturated ketones or precursors for such which have been considered together with other alpha,beta-unsaturated substances. The remaining 13 flavouring substances have originally been considered by EFSA in the FGE.51 (EFSA, 2008aj).

| <b>FGE</b> | <b>Opinion adopted by EFSA</b> | <b>Link</b>   | <b>No. of candidate substances</b> |
|------------|--------------------------------|---|------------------------------------|
| FGE.51     | 16 May 2007                    | <a href="http://www.efsa.europa.eu/en/efsajournal/doc/855.pdf">http://www.efsa.europa.eu/en/efsajournal/doc/855.pdf</a> | 13                                 |
| FGE.51Rev1 | 22 March 2012                  |   | 20                                 |

The present revision of FGE.51, FGE.51Rev1, includes the consideration of seven additional substances [FL-no: 07.034, 07.035, 07.098, 07.126, 07.129, 07.172 and 09.930].

Six of the seven additional substances [FL-no: 07.034, 07.035, 07.098, 07.126, 07.129 and 07.172] are alpha,beta-unsaturated ketones originally allocated to FGE.211 (EFSA, 2011e) and FGE.212 (EFSA, 2009ai). The seventh substance [FL-no: 09.930] is a precursor for such ketones originally allocated to FGE.211. The seven substances have been considered with respect to genotoxicity and the Panel concluded in FGE.211 (EFSA, 2011e) and FGE.212Rev1 (EFSA, 2011f) that the data available ruled out the concern for genotoxicity and accordingly the substances can be evaluated through the Procedure in this FGE.51Rev1. The information concerning genotoxicity of these seven substances is described in Section 3.3 and 3.4.

Since the publication of FGE.51, the EU production volume has been provided for the substance, [FL-no: 09.230] for which the evaluation could not be finalised in the previous version of this FGE, due to lack of these data. Based on the newly submitted EU production volume, the substance has already been evaluated in FGE.96<sup>4</sup> (EFSA, 2010al), but for the sake of completion, the information has also been included here as well.

<sup>4</sup> Consideration of 88 flavouring substances considered by EFSA for which EU production volumes / anticipated production volumes have been submitted on request by DG SANCO.



Finally, new information on the stereoisomeric composition has been provided for four substances [FL-no: 02.209, 07.045, 07.095 and 07.257] since the previous version of FGE.51 (EFSA, 2010a).

A search in open literature for the seven new substances did not provide any further data on toxicity or metabolism.

## **1. Presentation of the Substances in the JECFA Flavouring Group**

### **1.1. Description**

#### **1.1.1. JECFA Status**

The JECFA has evaluated at its 59<sup>th</sup> meeting a group of 25 flavouring substances consisting of alicyclic ketones, secondary alcohols and related esters (JECFA, 2002d; JECFA, 2003a).

#### **1.1.2. EFSA Considerations**

Two of the JECFA-evaluated substances are not in the Register (4-methyl cyclohexanone (JECFA no: 1104) and (E)-2-(2-octenyl) cyclopentanone (JECFA no: 1116)).

Seven of 10 alpha,beta-unsaturated ketones or precursors for such [FL-no: 07.034, 07.035, 07.098, 07.126, 07.129, 07.172 and 09.930] have been considered with respect to genotoxicity in FGE.211 (EFSA, 2011e) and FGE.212Rev1 (EFSA, 2011f), and the Panel concluded that the data available ruled out the concern for genotoxicity and accordingly the seven substances can be evaluated through the Procedure in this FGE.

For the remaining three substances [FL-no: 07.033, 07.094 and 07.112] considered with respect to genotoxicity in FGE.212Rev1, a final conclusion of genotoxic properties could not be reached and additional data were requested. Accordingly, these three substance will not be considered in this FGE.

This consideration will therefore deal with 20 JECFA-evaluated substances.

The Panel concluded that the 20 substances in the JECFA flavouring group of alicyclic ketones, secondary alcohols and related esters are structurally related to the group of secondary alicyclic saturated and unsaturated alcohols, ketones and esters with secondary alicyclic alcohol moieties evaluated by EFSA in Flavouring Group Evaluation 09, Revision 3 (FGE.09Rev3) (EFSA, 2011x).

## **1.2. Isomers**

### **1.2.1. Status**

Six of the substances have one chiral centre [FL-no: 07.045, 07.129, 07.172, 07.179, 07.180 and 07.257] and four substances have two or more chiral centres [FL-no: 02.209, 07.035, 07.095 and 09.930]. Two substances have possibility for cis/trans isomerism [FL-no: 07.034 and 07.257].

### **1.2.2. EFSA Considerations**

Adequate information on isomeric composition is available for all substances.

## **1.3. Specifications**

### **1.3.1. JECFA Status**

The JECFA specifications are available for all the 20 substances (JECFA, 2002d). See Table 1.

### **1.3.2. EFSA Considerations**

The available specifications are considered adequate for all the substances (See Section 1.2).

## 2. Intake Estimations

### 2.1. JECFA Status

For all the substances evaluated through the JECFA Procedure, intake data are available for the EU, see Table 3.1.

### 2.2. EFSA Considerations

Tonnage data are available for the EU allowing calculation of the intake estimates (MSDI). The Panel noted that since no use levels were submitted no mTAMDI values can be calculated.

## 3. Genotoxicity Data

### 3.1. Genotoxicity Studies – Text Taken<sup>5</sup> from the JECFA (JECFA, 2003a)

#### *In vitro*

Eight of the 13<sup>6</sup> alicyclic ketones, secondary alcohols and related esters have been tested for genotoxicity. Overall, negative results were reported in the standard assay for reverse mutation when various strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537 and TA1538) were incubated with up to 10000 microgram/plate of cyclohexanone [FL-no: 07.148] or isophorone [FL-no: 07.126], 2.5 - 2500 µg/plate of cyclopentanone [FL-no: 07.149], up to 4200 µg/plate of 2,2,6-trimethylcyclohexanone [FL-no: 07.045] or up to 3600 µg/plate of 2-hexylidenecyclopentan-1-one [FL-no: 07.034] or tetramethyl ethylcyclohexanone [FL-no: 07.035], with or without metabolic activation (Florin et al., 1980; Haworth et al., 1983; Wild et al., 1983; Mortelmans et al., 1986). In another test for reverse mutation with *S. typhimurium* TA98, TA100, TA1535 and TA1537 (only an abstract), cyclohexanone was reported to produce ‘a large number of revertants’ in TA98, with no further elaboration and no results for the other strains. The concentrations and test conditions used were not specified (Massoud et al., 1980).

Both cyclohexyl acetate [FL-no: 09.027] and cyclohexyl butyrate [FL-no: 09.230] gave negative results for mutation in *Bacillus subtilis* M45 (*rec*<sup>-</sup>) and H17 (*rec*<sup>+</sup>) (Oda et al., 1979; Yoo, 1986). Positive results were reported with cyclohexanone in an assay for forward mutation assay in *B. subtilis* (Massoud et al., 1980); however, as previously stated, no concentrations or test conditions were reported in the abstract.

The results for forward mutation in mouse lymphoma cells were generally negative with isophorone, with or without metabolic activation (NTP, 1986d; McKee et al., 1987; O'Donoghue et al., 1988). An increased mutation frequency was reported in L5178Y Tk<sup>+/-</sup> mouse lymphoma cells without metabolic activation at concentrations of 400 and 800 µg/ml. Isophorone was lethal at 1600 µg/ml (MacGregor et al., 1988a).

Cyclohexanone [FL-no: 07.148] at concentrations up to 980 microgram/ml induced chromosomal aberrations in human lymphocytes with or without metabolic activation (Collin, 1971; Lederer et al., 1971; Dyshlovoi et al., 1981). It did not induce chromosomal aberrations in Chinese hamster ovary cells at a concentration of 7.5 µl/ml, with or without metabolic activation (Aaron et al., 1985). Isophorone [FL-no: 07.126] gave equivocal results in Chinese hamster ovary cells. In one study, no chromosomal aberrations were induced with or without metabolic activation at concentrations up to 1600 µg/ml (Gulati et al., 1989), whereas in another study isophorone at a concentration of 1200 µg/ml without metabolic activation or at a concentration of 1500 µg/ml with metabolic activation induced chromosomal aberrations (Matsuoka et al., 1996); however, lower concentrations of 250 - 1000 µg/ml tested without metabolic activation did not. In an assay for sister chromatid exchange,

<sup>5</sup> The text is taken verbatim from the indicated reference source, but text related to substances not included in FGE.51 has been removed.

<sup>6</sup> The genotoxicity data available for the seven new substances are summarised in Sections 3.3. and 3.4.

cyclohexanone at a concentration of 7.5 µl/ml gave weakly positive results in Chinese hamster ovary cells in the absence of metabolic activation and negative results in the presence of metabolic activation (Aaron et al., 1985). Similarly, isophorone induced sister chromatid exchange in Chinese hamster ovary cells only when tested without metabolic activation at concentrations of 500 - 1000 µg/ml and then only after delayed harvesting due to the cytostatic effect of isophorone (Gulati et al., 1989). At lower concentrations tested without metabolic activation or at concentrations up to 1600 µg/mL tested with metabolic activation, isophorone did not induce sister chromatid exchange (NTP, 1986d; Gulati et al., 1989). In an assay for unscheduled DNA synthesis in rat hepatocytes, isophorone showed no sign of genotoxicity at concentrations up to 200 µl/ml (McKee et al., 1987; O'Donoghue et al., 1988).

#### *In vivo*

When cyclohexanone [FL-no: 07.148], 2-hexylidenecyclopentan-1-one [FL-no: 07.034], tetramethyl ethylcyclohexanone [FL-no: 07.035] or isophorone [FL-no: 07.126] was fed to adult *Drosophila melanogaster* for 3 days, no mutations were observed (Goncharova, 1970; Wild et al., 1983; Foureman et al., 1994). In addition, negative results were obtained when *D. melanogaster* were injected with a single dose of 12 500 µg of isophorone (Foureman et al., 1994).

There was no increase in the frequency of micronucleated polychromatic erythrocytes in the bone marrow of male or female CD-1 mice given isophorone [FL-no: 07.126] at a dose of 540 µg/kg bw by intraperitoneal injection (McKee et al., 1987; O'Donoghue et al., 1988) or in NMRI mice injected intraperitoneally with 2-hexylidenecyclopentan-1-one at a dose of 170, 330 or 500 mg/kg bw or tetramethyl ethylcyclopentenone at a dose of 180, 310 or 450 mg/kg bw (Wild et al., 1983).

#### *Conclusion on genotoxicity*

Cyclohexyl acetate [FL-no: 09.027], cyclohexyl butyrate [FL-no: 09.230], cyclopentanone [FL-no: 07.149], 2,2,6-trimethyl cyclohexanone [FL-no: 07.045] and tetramethyl ethylcyclohexanone (mixed isomers) [FL-no: 07.035], gave negative results in assays for genotoxicity *in vitro*. The results reported for the genotoxicity of cyclohexanone [FL-no: 07.148] and isophorone [FL-no: 07.126] are conflicting. Most of the assays were conducted before 1986, when the pH and ionic strength of test media were often not adequately maintained. Mammalian cells *in situ* rely on complex regulatory mechanisms to maintain homeostatic conditions, and those in culture are not equipped to respond to environmental changes; therefore, it is important that the culture media used in mammalian cell assays be maintained at a pH of approximately 6.8 - 7.5. A lower pH or changes in osmolality due to the test agents can give rise to false-positive results, especially when metabolic activation systems are added. Acidity facilitates the breakdown of the components of such systems into mutagenic agents (Brusick, 1986).

The equivocal results of the assays for genotoxicity with cyclohexanone *in vitro* can be interpreted in terms of physiochemical properties. Compounds that are structurally similar to cyclohexanone have excellent membrane permeability and hydrogen bonding potential (Slater, 1963; Slater, 1967; Moreland, 1994). When cyclohexanone and related substances are tested *in vitro*, they may induce membrane expansion, leading to multiple effects on membrane-related processes. Membrane expansion may increase cell volume and lipid storage vacuoles, block ionic conductance channels, limit the availability of ATP and alter ion fluxes and metabolite distribution between the cytoplasm and organelles. Given these physiochemical properties, it is highly unlikely that any consistent pattern of genotoxicity would result from a battery of assays in bacterial and mammalian cells.

Overall, the tests for genotoxicity yielded mainly negative results. Positive results were reported in mammalian cells at cytotoxic concentrations, usually in the absence of biotransformation enzymes. The results of assays *in vivo* were negative.

For a summary of *in vitro* / *in vivo* genotoxicity data considered by the JECFA, see Table 2.1. Some of the studies, however, have only been summarised in Tables 2.5 - 2.6.

### 3.2. Genotoxicity Studies - Text Taken<sup>7</sup> from EFSA FGE.09Rev3 (EFSA, 2011x)

#### *In vitro* / *in vivo*

Genotoxicity data are available for only three candidate substances cyclohexanol [FL-no: 02.070], cyclopentanol [FL-no: 02.135], methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520] and for nine supporting substances and one structurally related substances.

Cyclohexanol [FL-no: 02.070] was not genotoxic in two Ames tests and in an *in vivo* micronucleus assay, which are all considered as valid studies. However, the results of the *in vivo* study are of limited relevance, due to the lack of evidence that the substance did reach the bone marrow. Inconclusive results were reported in an *in vitro* chromosomal aberration assay with human leukocytes and negative results were reported in a dominant lethal mutations assay with *Drosophila melanogaster*; both studies were considered inadequate. Cyclopentanol [FL-no: 02.135] was studied in a valid Ames test. No mutagenicity was found.

A battery of *in vitro* and *in vivo* genotoxicity studies were conducted on methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520] including valid negative reverse mutation tests in *Escherichia coli* (Wagner and Klug, 2000) and *Salmonella typhimurium* (Thompson, 2000).

In a mouse lymphoma test, pre-dating GLP, a more than 2-fold increase of the mutant frequency over the solvent treated control values was found at the highest tested cytotoxic concentration of 300 µg/ml in the presence of metabolic activation, and at the two highest tested cytotoxic concentrations of 200 and 300 µg/ml, in the absence of metabolic activation. Only limited documentation is provided in the study report; together with the fact that several cultures were infected and a lack of a confirmatory test, it is impossible to assess the reliability of these results (Ross and Harris, 1979b).

No induction of forward mutations at the TK locus in L5178Y mouse lymphoma cells were found in a study performed in compliance with the current OECD test guidelines, both in the absence and in the presence of metabolic activation, up to and including cytotoxic concentrations (Cifone, 2001).

Methyl 3-oxo-2-pentyl-1-cyclopentylacetate was tested in a bone marrow micronucleus test in mice following a single intraperitoneal administration of 0, 280, 560 or 1120 mg/kg bw in corn oil. The study was performed in compliance with the current OECD test guidelines. The two highest doses chosen induced clear signs of toxicity; slight reductions (up to 12 %) in the ratio of polychromatic erythrocytes to total erythrocytes were found, indicating that the test material had reached the target cells. No increase in micronucleated cells was found in the groups treated with the test material. The positive control induced the expected increases (Gudi and Krsmanovic, 1998).

In an Unscheduled DNA Synthesis (UDS) study, the ability of methyl 3-oxo-2-pentyl-1-cyclopentylacetate to induce DNA repair was studied in isolated rat hepatocytes after administration *in vivo*. The study was performed in compliance with the current OECD Guideline 486 (OECD, 1997). Methyl 3-oxo-2-pentyl-1-cyclopentylacetate was administered to male Sprague-Dawley CD rats by intra-peritoneal injection in doses of 333.3 and 1000 mg/kg bw (the latter dose was the maximum tolerated dose) followed by liver perfusion at 2 or 16 hours after dosing. No marked increase in the incidence of UDS was observed at either dose level or perfusion time. Statistically significant differences were revealed in the positive control groups when compared to the negative control group and the test article (Durward, 2001).

Genotoxicity data are available for nine supporting substances [FL-no: 02.015, 02.062, 07.148, 07.176, 09.027, 09.215, 09.230, 07.149 and 07.045].

<sup>7</sup> The text is taken verbatim from the indicated reference source.

Cyclohexanone [FL-no: 07.148], structurally related to the alicyclic ketones and secondary alcohols in this FGE, was not mutagenic in an Ames test, considered to be valid. Negative and positive results were reported in several other *in vitro* studies at gene and chromosomal level, as well as a negative result in a sex-linked recessive lethal mutations in *D. melanogaster*. However, these studies were considered inadequate.

Menthol [FL-no: 02.015] gave negative results in an *in vitro* alkaline elution assay for detecting DNA single strand breaks in rat hepatocytes. With the same substance equivocal results in an *in vivo* host mediated mutation assay were observed at high dose levels and negative results in several Ames tests, a TK+/- mouse lymphoma assay, sister chromatid exchange (SCE) tests in Chinese hamster ovary (CHO) cells and human lymphocytes, and chromosomal aberration assays with human embryonic lung cells, human lymphocytes and CHO cells. Negative results were also reported in two *in vivo* micronucleus and chromosomal aberration assays. However, the results of these studies have a limited relevance, due to the lack of bone marrow toxicity. In addition, an *in vivo* dominant lethal assay was available, from which also negative results were obtained. *trans*-Menthone [FL-no: 07.176] was genotoxic in an Ames test and in a somatic mutation and recombination test (SMART) with *Drosophila*. The observed effects were not very pronounced. Further, *trans*-menthone is easily converted to menthol, which is estimated to be overall negative in genotoxicity tests.

Carveol and carvyl acetate [FL-no: 02.062 and 09.215] were tested in Ames test at various doses from 10 - 560 µg/plate in the *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535 and TA1537 with and without S9 mix in dimethyl sulphoxide. Positive and negative controls were used. No mutagenicity was observed (Mortelmans et al., 1986).

#### *Conclusion on genotoxicity*

Only for three of the candidate substances some genotoxicity data are available, and for these three mainly negative results were obtained. For the supporting substances mainly negative, but also some positive results were obtained. The positive results were obtained in poorly reported tests, or in tests, which are difficult to interpret with respect to their relevance for genotoxicity.

Overall, the genotoxic potential of this group of flavouring substances cannot be fully assessed as it is now. However, the data available do not indicate a genotoxic potential and therefore do not preclude their evaluation via the Procedure.

For a summary of *in vitro* / *in vivo* genotoxicity data considered by EFSA, see Table 2.2 and 2.3.

### **3.3. Genotoxicity Studies - Text Taken<sup>8</sup> from EFSA FGE.211 (EFSA, 2011e)**

The following text is relevant for two substances [FL-no: 07.034 and 09.930] in this revision of FGE.51. These substances were evaluated based on structural similarity 1(7),8-p-menthadien-2-yl acetate [FL-no: 09.930].

The Industry has submitted data concerning genotoxicity studies for the one representative substance for subgroup 2.5 of FGE.19 (FGE.211), 1(7),8-p-menthadien-2-yl acetate [FL-no: 09.930] (structurally related to 1(7),8-p-menthadien-2-one).

#### *In vitro data*

The newly available data comprise a bacterial reverse mutation assay and an *in vitro* micronucleus assay with human peripheral blood lymphocytes. The genotoxicity assays have been performed on a commercial mixture of the representative substance 1(7),8-p-menthadien-2-yl acetate and a positional isomer, carvyl acetate. Carvyl acetate can be hydrolysed followed by oxidation to carvone, which has been evaluated by EFSA in FGE.212 (EFSA, 2009ai) and NTP (NTP, 1990b) as non-genotoxic. The

<sup>8</sup> The text is taken verbatim from the indicated reference source



highest concentration of d-carvone that could be tested without cytotoxicity was 333 µg/plate (Mortelmans et al., 1986), i.e. the cytotoxicity was in the same range as observed for the mixture of 1(7),8-p-menthadien-2-yl acetate/carvyl acetate. The Panel concluded that testing the commercial mixture of 1(7),8-p-menthadien-2-yl acetate/carvyl acetate for genotoxicity allows the evaluation of the genotoxic potential of 1(7),8-p-menthadien-2-yl acetate. The concentrations reported in Table 2.4 (FGE.51Rev1) are for the mixture of substances.

#### *Bacterial Reverse Mutation Assay*

**1(7),8-p-menthadien-2-yl acetate/carvyl acetate** was tested for mutagenic activity according to OECD guideline 471 and in compliance with GLP (Beevers, 2010a). The test material exhibited a marked toxicity as indicated by thinning of the background lawn, reduced revertant counts and complete killing of test bacteria. However, the Panel considered the remaining number of concentrations without signs of toxicity sufficient to draw a conclusion on mutagenicity in this system (for details, see Table 2.4 of this FGE.51Rev1).

Overall, the Panel concluded that there was no evidence of mutagenic activity of 1(7),8-p-menthadien-2-yl acetate/carvyl acetate at concentrations up to those causing bactericidal effects.

#### *In vitro Micronucleus Test*

**1(7),8-p-menthadien-2-yl acetate/carvyl acetate** was tested for induction of micronuclei in human peripheral blood lymphocytes according to OECD guideline 487 and in compliance with GLP (Whitwell, 2010b). The Panel considered that acceptable levels of cytotoxicity as judged upon the replication index were achieved at the top concentrations (for details see Table 2.4 of this FGE.51Rev1).

Overall, the Panel concluded that no evidence of chromosomal damage or aneuploidy was observed by increased levels of micronucleated binucleate cells (MNBN) in the presence or absence of S9 metabolic activation.

A summary of the *in vitro* genotoxicity data is given in Table 2.4

#### *Discussion of Mutagenicity/Genotoxicity Data*

The commercial mixture of the representative substance 1(7),8-p-menthadien-2-yl acetate and a positional isomer, carvyl acetate, was tested for all three genetic endpoints: gene mutations, structural and numerical chromosomal aberrations. The test material did not induce gene mutations in bacteria and was not clastogenic and/or aneugenic in mammalian cells *in vitro*. Although this commercial mixture was cytotoxic at high concentrations, the remaining concentrations without signs of toxicity provide a valid data set.

#### *Conclusion*

The *in vitro* genotoxicity data on the commercial mixture of the representative substance 1(7),8-p-menthadien-2-yl acetate [FL-no: 09.930] and a positional isomer, carvyl acetate, do not indicate genotoxic potential. Accordingly the four substances in FGE.211 (subgroup 2.5 of FGE.19) would be of no safety concern with respect to genotoxicity.

### 3.4. Genotoxicity Studies - Text Taken<sup>9</sup> from EFSA FGE.212 (EFSA, 2009ai) and FGE.212Rev1 (EFSA, 2011f)

The following text is relevant for five substances [FL-no: 07.035, 07.098, 07.126, 07.129 and 07.172] in this revision of FGE.51. These substances were evaluated based on structural similarity with isophorone [FL-no: 07.126].

For tetramethyl ethylcyclohexenone (mixture of isomers) [FL-no: 07.035] one *in vitro* and one *in vivo* study are available and have been evaluated. Seven *in vitro* and three *in vivo* studies are available for 3,5,5 trimethylcyclohex-2-en-1-one [FL-no: 07.126] (isophorone).

3,5,5 Trimethylcyclohex-2-en-1-one [FL-no: 07.126] (isophorone) did not induce gene mutations in bacteria but it induced mutations in mammalian cells in a mouse lymphoma TK assay in the absence of metabolic activation (it was not tested in the presence of metabolic activation) (NTP, 1986d). No mutations in the MLTK assay were observed in a study of O'Donoghue et al. (O'Donoghue et al., 1988) at comparable concentrations. Isophorone induced chromosomal aberrations in Chinese hamster lung fibroblasts with and without metabolic activation (Matsuoka et al., 1996) and sister chromatid exchanges (SCE) in CHO cells without metabolic activation (Gulati et al., 1989). Chromosomal aberrations have not been observed in two other studies (Gulati et al., 1989; NTP, 1986d); however, the validity of the results was limited because the types of aberrations were not reported. Isophorone did not induce unscheduled DNA synthesis (UDS) in rat hepatocytes *in vitro*. *In vivo*, isophorone was tested negative in a sex-linked recessive lethal mutation assay in *Drosophila* (Fouremant et al., 1994) and in two micronucleus assays in mice (McKee et al., 1987; O'Donoghue et al., 1988). However, the *Drosophila* assay has only limited relevance and the micronucleus assays were of limited validity.

Negative results were also observed with tetramethyl ethylcyclohexenone [FL-no: 07.035] in bacteria, in a sex-linked recessive lethal mutation assay in *Drosophila* (Wild et al., 1983) and in a mouse micronucleus assay (Wild et al., 1983); however, there was a mixture of isomers tested and the studies were only of limited validity.

#### *Conclusion on Genotoxicity from FGE.212*

Isophorone [FL-no: 07.126 (3,5,5-trimethylcyclohex-2-en-1-one)] is genotoxic *in vitro* and since there is some evidence of carcinogenicity in male rats and equivocal evidence of carcinogenicity in male mice and since a non-threshold mechanism could not be excluded based on the data currently available, the Panel concluded that additional data are required for isophorone in order to clarify whether genotoxicity occurs *in vivo* and whether there is a threshold for the effects observed in the target organs in the long-term bioassays. Therefore, an *in vivo* Comet assay in F344/N rats covering these target organs is required in addition to an *in vivo* bone marrow assay with oral application.

Due to structural similarities and lack of data, the remaining substances cannot presently be evaluated through the Procedure [FL-no: 07.035, 07.098, 07.129 and 07.172]. Additional data on genotoxicity are requested for representative substances of this subgroup according to the opinion of the Panel on the Genotoxicity Test Strategy for Substances Belonging to Subgroups of FGE.19 (EFSA, 2008bb)

#### *Data submitted from Industry in reply to request for additional genotoxicity data in FGE.212*

Honma *et al.* (Honma et al., 1999a; Honma et al., 1999b) found that isophorone did not clearly induce mutations in the mouse lymphoma assay (MLA) following 3 hour treatments, but observed that it was mutagenic after 24 hour treatments in the absence of S9. Although only graphs are plotted, it seems that increases in mutation frequency (MF) that exceeded the Global Evaluation Factor (GEF) occurred at around 1250 - 1500 µg/ml where toxicity (by relative survival) reached 70 - 90 %.

<sup>9</sup> The text is taken verbatim from the indicated reference source, but text related to substances not included in FGE.51 has been removed.



The NTP conducted a mouse bone marrow chromosomal aberration (CA) study on isophorone. Groups of 8 male B6C3F1 mice (larger group sizes than required by OECD) were dosed i.p. with isophorone at 125, 250 and 500 mg/kg bw. The standard protocol for *in vivo* CA is not given on the NTP website. However, based on Shelby and Witt (Shelby and Witt, 1995), animals should have been sampled at 17 hours and, if negative, also at 36 hours. The data on the NTP website are only for bone marrow sampled at 36 hours. It is therefore possible that a 17 hours sample was also taken, and found to be negative, but the data have not been posted. Fifty cells per animal were scored for CA and no increases in CA were seen. No measures of toxicity were recorded, but i.p. dosing should have guaranteed systemic exposure. The control CA frequency was normal (2.75 %) and the positive control (dimethylbenzanthracene) produced a significant response in CA frequency.

A DNA binding study was conducted in which F344-rats and B6C3F1-mice (the strains used in the NTP carcinogenicity study) were exposed to isophorone (Thier et al., 1990). Animals of both sexes were dosed once or five times by gavage with 500 mg/kg bw of unlabelled isophorone spiked with [1,3,5-<sup>14</sup>C]-isophorone (specific activity: 52 mCi per mmol, 1.92 GBq per mmol). An additional group of acute dosed male rats received undiluted <sup>14</sup>C-isophorone for increased sensitivity. Rats and mice were maintained for 24 hours in closed metabolic cages. Twenty four hours after exposure, livers and kidneys (the tumour target tissues) were removed from the animals. DNA was isolated through hydroxyapatite chromatography and radioactivity was measured by liquid scintillation counting. No positive controls were included. Also no untreated controls were included, but, except for the liver sample of one mouse in the five times dose group, radioactivity values were within 2σ of background (6 dpm). Radioactivity values therefore did not indicate significant attachment of radioactivity to DNA. From these results it can be concluded that neither isophorone nor its metabolites bind covalently to DNA.

In addition, a report by Morishita *et al.* (Morishita et al., 1997b) submitted to EPA (EPA, 1997), is relevant and appears to have been previously submitted only as an abstract. This study was designed to investigate whether isophorone and/or α2μ-globulin<sup>10</sup> might be involved in the induction of preputial gland tumours in F-344 rats (10/sex/dose group). A series of experiments was performed in order to study several parameters including:

- binding of isophorone to DNA of kidney and preputial gland. Groups of 10 male rats were dosed by gavage with 500 mg/kg of [<sup>14</sup>C]-isophorone (specific activity 14.65 mCi/mmol; 100 μCi/animal). Positive control animals were dosed with <sup>3</sup>H-labeled methyl nitrosourea.
- DNA adduct detection by <sup>32</sup>P-postlabeling in young adult male and female rats (7 per group) dosed by gavage with 0, 250 or 500 mg/kg isophorone for five days.

Extraction of preputial gland and kidney DNA from rats treated with single 500 mg/kg labeled doses yielded no evidence of isophorone binding to DNA, whereas the positive control showed significant binding to DNA of preputial gland and kidney. These negative results with isophorone were confirmed in the <sup>32</sup>P-postlabeling assays.

#### *Discussion of the additional data*

Conflicting results were reported in two valid studies with the mouse lymphoma assay (MLA): one negative (O'Donoghue et al., 1988) and one positive (NTP, 1986d) at comparable concentrations. Mixed results were also reported in two studies of limited validity: one negative (Honma et al., 1999a) and one positive (Honma et al., 1999b). Another negative result was reported in a study (McKee et al., 1987), the validity of which cannot be evaluated. In the light of the clearly negative results in two valid bacterial gene mutation tests (Ames test) and in a valid Sex Linked Recessive Lethal Mutations test (SLRL) in *Drosophila*, and taking into account the lack of specificity and high sensitivity of the MLA, overall the results presently available are considered of questionable relevance. The Panel

<sup>10</sup> Since interaction with α2μ-globulin is not of direct relevance for the evaluation of genotoxic potential, this information is omitted from this study summary.

agrees that isophorone demonstrates some genotoxic activity *in vitro* but that the new data demonstrate lack of clastogenicity *in vivo*. In addition, the new DNA-binding data from two separate studies provide convincing evidence that isophorone does not induce tumours via a genotoxic mechanism. On the basis of these data it may be argued that there is no need to perform further *in vivo* genotoxicity studies such as the Comet assay or bone marrow micronucleus test. Thus, based on the data available the Panel concluded in FGE.212Rev1 that there is no concern with respect to genotoxicity of isophorone.

A summary of the *in vitro* and *in vivo* genotoxicity data from FGE.212Rev1 is given in Tables 2.5 and 2.6.

### 3.5. EFSA Considerations

Data not available for the JECFA at the time of evaluation (59<sup>th</sup> meeting) for cyclohexanone [FL-no: 07.148] have been considered by EFSA. Results from *in vitro* genotoxicity studies with cyclohexanone, carried out by NTP, have been published on the NTP website (NTP, 2007). From the technical information also provided there, it can be concluded that the tests by NTP are reliable. A set of Ames tests with *Salmonella* strains TA98, TA100, TA1535 and TA1537) and a study with mouse lymphoma cells (L5178Y; tk<sup>+</sup>/), including cloning efficiency and colony sizing provided convincingly negative results. The tests were carried out with and without metabolic activation at cyclohexanone levels up to 10000 microg/plate in the Ames tests and up to 5000 microg/ml in the mouse lymphoma assay. For a summary of these studies see Table 2.7.

The Panel noted that cyclohexanone has also been studied in long term carcinogenicity studies in mice (up to 6.2 g/kg bw/day) and rats (up to 0.65 g/kg bw/day) (Lijinsky and Kovatch, 1986). The substance was tested up to the maximum tolerated dose levels and the overall conclusion from these studies was that cyclohexanone is not carcinogenic. In an evaluation of these studies the IARC concluded that the substance was not classifiable as to its carcinogenicity to humans (IARC, 1989).

For seven candidate substances [FL-no: 07.034, 07.035, 07.098, 07.126, 07.129, 07.172 and 09.930] it has been concluded in FGE.211 and FGE.212Rev1, that a concern for genotoxicity, indicated by the presence of a structural alert, could be ruled out based on experimental data for representative substances.

Based on these results the Panel concluded that the data available do not preclude evaluation of the 20 JECFA evaluated alicyclic ketones, secondary alcohols and related esters through the Procedure.

## 4. Application of the Procedure

### 4.1. Application of the Procedure to 20 Alicyclic Ketones, Secondary Alcohols or Related Esters Evaluated by the JECFA (JECFA, 2003a):

According to the JECFA six of the substances belong to structural class I and 14 to structural class II using the decision tree approach presented by Cramer *et al.* (Cramer *et al.*, 1978).

The JECFA concluded all 20 alicyclic ketones, secondary alcohols or related esters at step A3 in the JECFA Procedure – i.e. the substances are expected to be metabolised to innocuous products (step 2) and the intakes for all substances are below the thresholds for structural classes I and II (step A3).

In conclusion, the JECFA evaluated all 20 substances as to be of no safety concern at the estimated levels of intake as flavouring substances based on the MSDI approach.

The evaluations of the 20 substances are summarised in Table 3.1: Summary of Safety Evaluation of Alicyclic Ketones, Secondary Alcohols or Related Esters (JECFA, 2003a).

#### **4.2. Application of the Procedure to 17 Secondary Alicyclic Saturated and Unsaturated Alcohols, Ketones and Esters Containing Secondary Alicyclic Alcohols by EFSA in FGE.09Rev3 (EFSA, 2011x):**

Seventeen flavouring substances were evaluated in FGE.09Rev3. Thirteen substances are classified into structural class I, three into structural class II and one into structural class III using the decision tree approach presented by Cramer *et al.* (Cramer *et al.*, 1978).

Sixteen substances were concluded at step A3 using the EFSA Procedure – i.e. the substances are expected to be metabolised to innocuous products (step 2) and the estimated daily intakes for 15 substances are below the thresholds of concern for their structural classes (step A3).

For one substance methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520] the estimated daily intake exceeds the threshold of concern for structural class II and since the substance is not endogenous the substance proceeds to step A5.

A 90 day study in rats has been performed for [FL-no: 09.520] from which a No Observed Adverse Effect Level (NOAEL) of 100 mg/kg body weight (bw)/day could be derived. This NOAEL provides a margin of safety of nearly  $10^4$  compared to the daily intake of 0.013 mg/kg bw/day for methyl 3-oxo-2-pentyl-1-cyclopentylacetate. Therefore, [FL-no: 09.520] does not pose a safety concern when used at estimated levels of intake, based on the MSDI approach, as a flavouring substance

One flavouring substance [FL-no: 07.207] was not expected to be metabolised to innocuous products and was therefore evaluated via the B-side in the EFSA Procedure. The estimated intake is below the threshold, but no adequate No Observed Adverse Effect Level (NOAEL) could be provided for the substance or a structurally related substance – therefore additional data are required for this substance.

In conclusion, the Panel considered that 16 of the substances evaluated through the Procedure were of no safety concern at the estimated levels of intakes based on the MSDI approach. For one substance additional data were required.

The stepwise evaluations of the 17 substances are summarised in Table 3.2: Summary of Safety Evaluation Applying the Procedure (EFSA, 2011x).

#### **4.3. EFSA Considerations**

The Panel agrees with the application of the Procedure as performed by the JECFA for the 20 substances in the group of alicyclic ketones, secondary alcohols and related esters.

### **5. Conclusions**

The JECFA has evaluated a group of 25 flavouring substances consisting of alicyclic ketones, secondary alcohols and related esters at its 59<sup>th</sup> meeting. Two of the JECFA-evaluated substances are not in the Register (4-methyl cyclohexanone (JECFA no: 1104) and (E)-2-(2-octenyl) cyclopentanone (JECFA no: 1116)). Ten of the remaining 23 JECFA-evaluated substances are alpha,beta-unsaturated ketones or precursors for such, which structural property has been recognised as a structural alert for genotoxicity. Seven of these 10 candidate substances [FL-no: 07.034, 07.035, 07.098, 07.126, 07.129, 07.172 and 09.930] have been considered with respect to genotoxicity in FGE.211 (EFSA, 2011e) or FGE.212Rev1 (EFSA, 2011f), and the Panel concluded that the data available ruled out the concern for genotoxicity and accordingly these seven substances can be evaluated through the Procedure. For the remaining three substances [FL-no: 07.033, 07.094 and 07.112] considered with respect to genotoxicity in FGE.212Rev1 a final conclusion of genotoxic properties could not be reached and additional data were requested. These three substances will therefore not be considered in this revision of FGE. 51. This consideration therefore deals with 20 JECFA-evaluated substances.

The Panel concluded that the 20 substances in the JECFA group of alicyclic ketones, secondary alcohols and related esters are structurally related to the group of 17 secondary alicyclic saturated and unsaturated alcohols, ketones and esters containing secondary alicyclic alcohols evaluated in the Flavouring Group Evaluation 09, Revision 3 (FGE.09Rev3).

The Panel agrees with the application of the Procedure as performed by the JECFA for the 20 substances considered in this FGE.

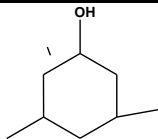
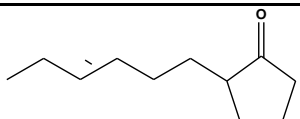
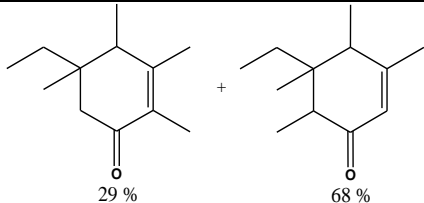
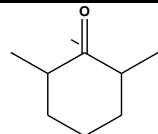
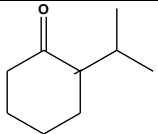
For all substances evaluated through the Procedure use levels are needed to calculate the mTAMDI in order to identify those flavouring substances that need more refined exposure assessment and to finalise the evaluation.

In order to determine whether the conclusion for the JECFA-evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity tests are available for all 20 substances.

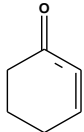
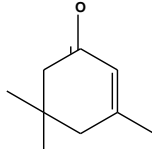
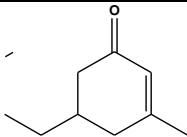
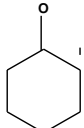
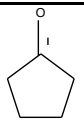
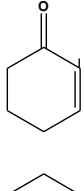
For all 20 JECFA-evaluated alicyclic ketones, secondary alcohols and related esters [FL-no: 02.209 07.034, 07.035, 07.045, 07.095, 07.098, 07.126, 07.129, 07.148, 07.149, 07.172, 07.179, 07.180, 07.257, 09.027, 09.140, 09.160, 09.230, 09.464 and 09.930] the Panel agrees with the JECFA conclusion, “No safety concern at estimated levels of intake as flavouring substance” based on the MSDI approach.

**TABLE 1: SPECIFICATION SUMMARY**

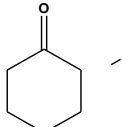
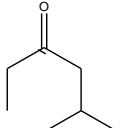
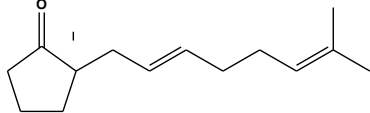
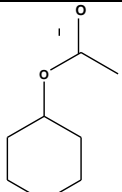
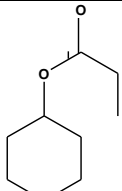
**Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of Alicyclic Ketones, Secondary Alcohols and Related Esters (JECFA, 2002d)**

| FL-no<br>JECFA-no | EU Register name                                   | Structural formula  | FEMA no<br>CoE no<br>CAS no | Phys.form<br>Mol.formula<br>Mol.weight                | Solubility 1)<br>Solubility in ethanol<br>2) | Boiling point, °C 3)<br>Melting point, °C<br>ID test<br>Assay minimum | Refrac. Index 4)<br>Spec.gravity 5) | EFSA comments   |
|-------------------|--|---|-----------------------------|---|--|---|-------------------------------------|---|
| 02.209<br>1099    | 3,3,5-Trimethylcyclohexan-1-ol                     |    | 3962<br>116-02-9            | Solid<br>C <sub>9</sub> H <sub>18</sub> O<br>142.24   | Insoluble<br>Soluble                         | 193-196<br>30-34<br>IR MS<br>98 %                                     | n.a.<br>n.a.                        | Racemate (EFFA, 2010a).   |
| 07.034<br>1106    | 2-Hexylidenecyclopentan-1-one                      |    | 2573<br>167<br>17373-89-6   | Liquid<br>C <sub>11</sub> H <sub>18</sub> O<br>166.26 | Insoluble<br>Miscible                        | 240<br>NMR<br>98 %  | 1.477-1.484<br>0.907-0.914          | Mixture E/Z (50/50) (EFFA, 2012b).  |
| 07.035<br>1111    | Tetramethylethylcyclohexenone (mixture of isomers) |    | 3061<br>168<br>17369-60-7   | Liquid<br>C <sub>12</sub> H <sub>20</sub> O<br>180.29 | Slightly soluble<br>Miscible                 | 113-115<br>NMR<br>97 %  | 1.485-1.490<br>0.927-0.934          | Mixture of 5-ethyl-2,3,4,5-tetramethyl-2-cyclohexen-1-one and 5-ethyl-3,4,5,6-tetramethyl-2-cyclohexen-1-one. The predominant constituent is 5-ethyl-3,4,5,6-tetramethyl-2-cyclohexen-1-one. Mixture of diastereoisomers in approximately equal ratios (EFFA, 2012b). |
| 07.045<br>1108    | 2,2,6-Trimethylcyclohexanone                       |   | 3473<br>686<br>2408-37-9    | Liquid<br>C <sub>9</sub> H <sub>16</sub> O<br>140.23  | Insoluble<br>Miscible                        | 178-179<br>NMR<br>99 %  | 1.443-1.449<br>0.900-0.907          | Racemate (EFFA, 2010a).   |
| 07.095<br>1109    | 2-(sec-Butyl)cyclohexanone                         |  | 3261<br>11044<br>14765-30-1 | Liquid<br>C <sub>10</sub> H <sub>18</sub> O<br>154.25 | Insoluble<br>Miscible                        | 76-78<br>NMR<br>94 %  | 1.454-1.461<br>0.911-0.917          | Mixture of diastereoisomers, approx. 25 % of each (EFFA, 2012b).<br>Min assay 94 % secondary comp. 2-isobutyl cyclohexanone 2-2.5 % (EFFA, 2010a).  |

**Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of Alicyclic Ketones, Secondary Alcohols and Related Esters (JECFA, 2002d)**

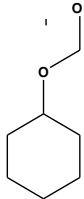
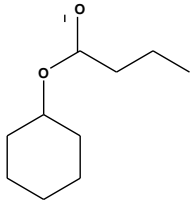
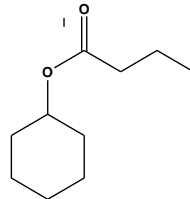
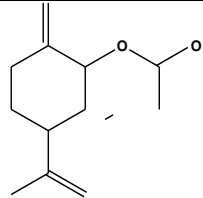
| FL-no<br>JECFA-no | EU Register name                     | Structural formula  | FEMA no<br>CoE no<br>CAS no | Phys.form<br>Mol.formula<br>Mol.weight                | Solubility 1)<br>Solubility in ethanol<br>2) | Boiling point, °C 3)<br>Melting point, °C<br>ID test<br>Assay minimum | Refrac. Index 4)<br>Spec.gravity 5) | EFSA comments           |
|-------------------|--------------------------------------|---|-----------------------------|---|--|---|-------------------------------------|-------------------------|
| 07.098<br>1107    | 3-Methylcyclohex-2-en-1-one          |    | 3360<br>11134<br>1193-18-6  | Liquid<br>C <sub>7</sub> H <sub>10</sub> O<br>110.16  | Miscible<br>Miscible                         | 199-200<br><br>NMR<br>98 %  | 1.490-1.498<br>0.967-0.972          |                         |
| 07.126<br>1112    | 3,5,5-Trimethylcyclohex-2-en-1-one   |    | 3553<br>11918<br>78-59-1    | Liquid<br>C <sub>9</sub> H <sub>14</sub> O<br>138.21  | Slightly soluble<br>Miscible                 | 213-215<br><br>NMR<br>97 %  | 1.474-1.481<br>0.919-0.927          |                         |
| 07.129<br>1113    | 3-Methyl-5-propylcyclohex-2-en-1-one |    | 3577<br><br>3720-16-9       | Liquid<br>C <sub>10</sub> H <sub>16</sub> O<br>152.23 | Insoluble<br>Miscible                        | 242-244<br><br>NMR<br>95 %  | 1.481-1.486<br>0.924-0.928          | Racemate (EFFA, 2012b). |
| 07.148<br>1100    | Cyclohexanone                        |    | 3909<br>11047<br>108-94-1   | Liquid<br>C <sub>6</sub> H <sub>10</sub> O<br>98.14   | Miscible                                     | 154-156<br><br>IR NMR MS<br>99 %                                      | 1.447-1.453<br>0.947-0.950          |                         |
| 07.149<br>1101    | Cyclopentanone                       |   | 3910<br>11050<br>120-92-3   | Liquid<br>C <sub>5</sub> H <sub>8</sub> O<br>84.12    | Miscible                                     | 130-131<br><br>IR NMR MS<br>99 %                                      | 1.432-1.438<br>0.950-0.960          |                         |
| 07.172<br>1110    | 4-Isopropylcyclohex-2-en-1-one       |  | 3939<br>11127<br>500-02-7   | Liquid<br>C <sub>9</sub> H <sub>14</sub> O<br>138.21  | Insoluble<br>Miscible                        | 198<br><br>NMR<br>97 %  | 1.481-1.490<br>0.930-0.950          | Racemate (EFFA, 2012b). |

**Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of Alicyclic Ketones, Secondary Alcohols and Related Esters (JECFA, 2002d)**

| FL-no<br>JECFA-no | EU Register name                               | Structural formula  | FEMA no<br>CoE no<br>CAS no | Phys.form<br>Mol.formula<br>Mol.weight                            | Solubility 1)<br>Solubility in ethanol<br>2) | Boiling point, °C 3)<br>Melting point, °C<br>ID test<br>Assay minimum | Refrac. Index 4)<br>Spec.gravity 5) | EFSA comments  |
|-------------------|--|---|-----------------------------|---|--|---|-------------------------------------|--|
| 07.179<br>1102    | 2-Methylcyclohexanone                          |    | 3946<br>583-60-8            | Liquid<br>C <sub>7</sub> H <sub>12</sub> O<br>112.17              | Insoluble<br>Miscible                        | 163-163<br>IR NMR MS<br>96 %  | 1.444-1.450<br>0.924-0.926          | Racemate.  |
| 07.180<br>1103    | 3-Methylcyclohexanone                          |    | 3947<br>591-24-2            | Liquid<br>C <sub>7</sub> H <sub>12</sub> O<br>112.17              | Insoluble<br>Miscible                        | 169-170<br>IR NMR MS<br>97 %  | 1.440-1.450<br>0.914-0.919          | Racemate.  |
| 07.257<br>1117    | 2-(3,7-Dimethyl-2,6-octadienyl) cyclopentanone |    | 3829<br>68133-79-9          | Liquid<br>C <sub>15</sub> H <sub>24</sub> O<br>220.35             | Insoluble<br>Miscible                        | 130 (4 hPa)<br>NMR MS<br>95 %   | 1.482-1.489<br>0.911-0.916          | Racemic mixture of (E)- and (Z)-isomers (EFFA, 2010a).<br>The double bond occurs mainly as E-isomer (at least 80 % E and max. 20 % Z) (EFFA, 2012b). |
| 09.027<br>1093    | Cyclohexyl acetate                             |   | 2349<br>217<br>622-45-7     | Liquid<br>C <sub>8</sub> H <sub>14</sub> O <sub>2</sub><br>142.19 | Insoluble<br>Miscible                        | 175-177<br>NMR<br>98 %  | 1.436-1.443<br>0.971-0.978          |  |
| 09.140<br>1097    | Cyclohexyl propionate                          |  | 2354<br>421<br>6222-35-1    | Liquid<br>C <sub>9</sub> H <sub>16</sub> O <sub>2</sub><br>156.23 | Insoluble<br>Miscible                        | 193<br>NMR<br>97 %  | 1.439-1.446<br>0.969-0.974          |  |



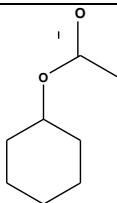
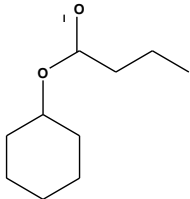
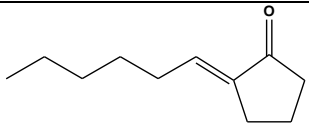
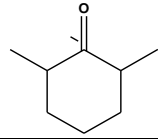
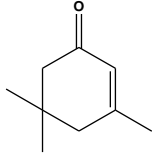

**Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of Alicyclic Ketones, Secondary Alcohols and Related Esters (JECFA, 2002d)**

| FL-no<br>JECFA-no | EU Register name  | Structural formula   | FEMA no<br>CoE no<br>CAS no | Phys.form<br>Mol.formula<br>Mol.weight                             | Solubility 1)<br>Solubility in ethanol<br>2) | Boiling point, °C 3)<br>Melting point, °C<br>ID test<br>Assay minimum | Refrac. Index 4)<br>Spec.gravity 5) | EFSA comments   |
|-------------------|---|--|-----------------------------|--|--|---|-------------------------------------|---|
| 09.160<br>1095    | Cyclohexyl formate  |   | 2353<br>498<br>4351-54-6    | Liquid<br>C <sub>7</sub> H <sub>12</sub> O <sub>2</sub><br>128.17  | Insoluble<br>Miscible                        | 162-163<br><br>NMR<br>97 %  | 1.439-1.445<br>1.052-1.060          |   |
| 09.230<br>1094    | Cyclohexyl butyrate   |   | 2351<br>2082<br>1551-44-6   | Liquid<br>C <sub>10</sub> H <sub>18</sub> O <sub>2</sub><br>170.25 | Practically insoluble<br>Miscible            | 212<br><br>NMR<br>98 %  | 1.439-1.451<br>0.953-0.959          |   |
| 09.464<br>1096    | Cyclohexyl isovalerate  |   | 2355<br>459<br>7774-44-9    | Liquid<br>C <sub>11</sub> H <sub>20</sub> O <sub>2</sub><br>184.28 | Insoluble<br>Miscible                        | 58-62<br><br>NMR<br>95 %  | 1.439-1.445<br>0.945-0.952          |   |
| 09.930<br>1098    | 1(7),8-p-Menthadien-2-yl<br>acetate (mixture of (E) and (Z)<br>isomers) |  | 3848<br><br>71660-03-2      | Liquid<br>C <sub>12</sub> H <sub>18</sub> O <sub>2</sub><br>194.27 | Insoluble<br>Miscible                        | 77-79 (0.1 hPa)<br><br>IR NMR MS<br>95 %                              | 1.473-1.479<br>0.964-0.970          | Mixtures of<br>diastereoisomers (25 % of<br>each) (EFFA, 2012b).<br>Registername to be<br>changed to Cyclohexyl, 2-<br>methylene-5-(1-<br>methylethenyl) acetate. |

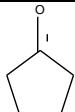
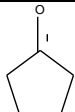
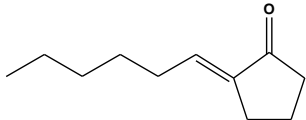
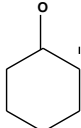
- 1) Solubility in water, if not otherwise stated.
- 2) Solubility in 95 % ethanol, if not otherwise stated.
- 3) At 1013.25 hPa, if not otherwise stated.
- 4) At 20°C, if not otherwise stated.
- 5) At 25°C, if not otherwise stated.

TABLE 2: GENOTOXICITY DATA

Table 2.1: Summary of Genotoxicity Data for Alicyclic Ketones, Secondary Alcohols and Related Esters (JECFA, 2003a)

| FL-no<br>JECFA-<br>no | EU Register name<br>JECFA name     | Structural formula  | End-point              | Test system   | Maximum<br>concentration  | Results               | Reference               |
|-----------------------|------------------------------------|---|------------------------|---|---|-----------------------|-------------------------|
| <i>In vitro</i>       |                                    |   |                        |   |   |                       |                         |
| 09.027<br>1093        | Cyclohexyl acetate                 |    | DNA damage             | <i>B. subtilis</i> H17( <i>rec</i> <sup>+</sup> ),<br>M45 ( <i>rec</i> <sup>-</sup> ) | 19 µg <sup>d</sup> /disc  | Negative <sup>a</sup> | (Yoo, 1986)             |
| 09.230<br>1094        | Cyclohexyl butyrate                |    | DNA damage             | <i>B. subtilis</i> H17( <i>rec</i> <sup>+</sup> ),<br>M45 ( <i>rec</i> <sup>-</sup> ) | 19 µg <sup>d</sup> /plate   | Negative <sup>a</sup> | (Oda et al., 1979)      |
| 07.034<br>1106        | 2-hexyldenecyclopentan-1-one       |   | Reverse mutation       | <i>S. typhimurium</i> TA98,<br>TA100, TA1535,<br>TA1537, TA1538                       | 5 concentrations, up to<br>cytotoxicity or max<br>36000 µg/plate. | Negative <sup>a</sup> | Wild et al., 1983.      |
| 07.045<br>1108        | 2,2,6-Trimethylcyclohexanone       |  | Reverse mutation       | <i>S. typhimurium</i> TA98,<br>TA100, TA1535,<br>TA1537                               | 4.2 - 3600 µg <sup>d</sup> /plate                                 | Negative <sup>a</sup> | (Florin et al., 1980)   |
| 07.126                | 3,5,5-Trimethylcyclohex-2-en-1-one |  | Foreward mutation test | Mouse lymphoma<br>L5178Y Tk <sup>+/+</sup> cells                                      | 0 – 1600 µg/ml  | Positive <sup>b</sup> | MacGregor et al., 1988a |
| 07.148<br>1100        | Cyclohexanone                      |  | Reverse mutation       | <i>S. typhimurium</i> TA98,<br>TA100, TA1535,<br>TA1537                               | 33 - 10 000 µg <sup>d</sup> /plate                                | Negative <sup>a</sup> | (Haworth et al., 1983)  |

**Table 2.1: Summary of Genotoxicity Data for Alicyclic Ketones, Secondary Alcohols and Related Esters (JECFA, 2003a)**

| FL-no<br>JECFA-<br>no | EU Register name<br>JECFA name | Structural formula   | End-point                            | Test system                                       | Maximum<br>concentration                                       | Results  | Reference                |
|-----------------------|--------------------------------|--|--------------------------------------|---|--|--|--------------------------|
|                       |                                |   | Reverse mutation                     | <i>S. typhimurium</i> TA98, TA100, TA1535, TA1537 | 2.9 - 2900 µg <sup>d</sup> /plate                              | Negative <sup>a</sup>                          | (Florin et al., 1980)    |
|                       |                                |  | Chromosomal                          | Chinese hamster ovary cells aberration            | 7.5 µl/ml  | Negative <sup>a</sup>                          | (Aaron et al., 1985)     |
|                       |                                |  | Chromosomal                          | Human lymphocytes aberration                      | 9.8 - 980 µg <sup>d</sup> /ml                                  | Positive <sup>a</sup>                          | (Lederer et al., 1971)   |
|                       |                                |  | Chromosomal                          | Human lymphocytes aberration                      | 0.005 - 0.1 µg <sup>d</sup> /ml                                | Positive <sup>a</sup>                          | (Dyshlovoi et al., 1981) |
|                       |                                |  | Sister chromatid exchange            | Chinese hamster ovary cells                       | 7.5 µl/ml  | Negative <sup>b</sup><br>Positive <sup>c</sup> | (Aaron et al., 1985)     |
| 07.149<br>1101        | Cyclopentanone                 |   | Reverse mutation                     | <i>S. typhimurium</i> TA98, TA100, TA1535, TA1537 | 2.5 - 2500 µg <sup>d</sup> /plate                              | Negative <sup>a</sup>                          | (Florin et al., 1980)    |
| <b><i>In vivo</i></b> |                                |  |                                      |   |  |  |                          |
| 07.034<br>1106        | 2-hexylidenecyclopentan-1-one  |   | Sex-linked recessive lethal mutation | <i>D. melanogaster</i>                            | 10 mM  | Negative                                       | Wild et al., 1983        |
|                       |                                |  | Micronucleus assay                   | NMRI mice (4/group)                               | 0, 166, 333, 500 mg/kg bw; single dose, 30 hrs expression time | Negative                                       | Wild et al., 1983        |
| 07.148<br>1100        | Cyclohexanone                  |  | Sex-linked recessive lethal mutation | <i>D. melanogaster</i>                            | 0.1 ml/100 ml  | Negative                                       | (Goncharova, 1970)       |

<sup>a</sup> With and without metabolic activation.

<sup>b</sup> Without metabolic activation.

<sup>c</sup> With metabolic activation.

<sup>d</sup> In the original JECFA report the figures for Maximum concentration were written as “mg”. This is a mistake by the JECFA as the concentration in the original references is reported in “µg”. Therefore “mg” has been replaced by “µg”.

**Table 2.2: GENOTOXICITY (*in vitro*) EFSA / FGE.09Rev3 (EFSA, 2011x) (substances in brackets are JECFA-evaluated substances)**

| Chemical Name [FL-no] | Test system                      | Test Object   | Concentration   | Result                | Reference  | Comments   |
|-----------------------|----------------------------------|---|---|-----------------------|--|--|
| (Menthol [02.015])    | Ames test                        | <i>S. typhimurium</i> TA92, TA94, TA98, TA100, TA1535, TA1537 | 0, and 6 concentrations up to 5000 µg/plate                               | Negative <sup>1</sup> | (Ishidate et al., 1984)                            | d,l-Menthol was used. The study is considered valid.   |
|                       | Ames test (preincubation method) | <i>S. typhimurium</i> TA97, TA98, TA100, TA1535               | 3 - 666 µg/plate  | Negative <sup>1</sup> | (Zeiger et al., 1988)                              | d,l-Menthol was used. The study is considered valid.   |
|                       | Ames test                        | <i>S. typhimurium</i> TA98, TA100, TA2637                     | 0, 5 - 500 µg/plate   | Negative <sup>1</sup> | (Nohmi et al., 1985)                               | d,l-Menthol was tested. The highest concentrations were cytotoxic. The study is considered valid.  |
|                       | Ames test                        | <i>S. typhimurium</i> TA98, TA100, TA2637                     | 0, 20 - 500 µg/plate  | Negative <sup>1</sup> | (Nohmi et al., 1985)                               | l-Menthol was tested. The highest concentrations were cytotoxic. The study is considered valid.  |
|                       | Ames test                        | <i>S. typhimurium</i> TA98, TA100, TA1535, TA1537             | 0, 6.4, 32, 160, and 800 µg/plate   | Negative <sup>1</sup> | (Andersen and Jensen, 1984b)                       | No indication of which enantiomer was used. In the absence of metabolic activation, the highest concentration was cytotoxic. The study is considered valid.  |
|                       | Ames test                        | <i>E. coli</i> WP2 <i>uvrA</i> (Trp <sup>-</sup> )            | 100 - 800 µg/plate  | Negative              | (Yoo, 1986)  | l-Menthol was used. The article is not in English. The validity of the study cannot be evaluated. It is unclear whether metabolic activation or a control group was used.  |
|                       | Ames test                        | <i>S. typhimurium</i> TA97A, TA98, TA100, TA102               | 0, 5 - 800 µg/plate   | Negative <sup>1</sup> | (Gomes-Carneiro et al., 1998)                      | (-)-Menthol was used. The range of concentrations tested varied between the different strains. Cytotoxicity was observed with the highest concentrations tested with TA97A and, in the presence of metabolic activation, the highest concentration tested with TA102. The study is considered valid.                     |
|                       | Rec assay                        | <i>B. subtilis</i> H17, M45                                   | Up to 10000 µg/disk   | Positive              | (Yoo, 1986)  | l-Menthol was used. Inhibition zone for rec- and rec+ was 42 and 23 mm, respectively. The article is not in English. It is not clear from the study whether metabolic activation, or a control group was used. The validity of this study cannot be assessed. The method ( <i>rec</i> -assay) has poor predictive value. |
|                       | Rec assay                        | <i>B. subtilis</i> H17, M45                                   | 20 µg/disk  | Negative              | (Oda et al., 1979)                                 | l-Menthol was used. The article is not in English. Only one concentration level is mentioned at a table. No data on metabolic activation or control group. The validity of this study cannot be evaluated. The method ( <i>rec</i> -assay) has poor predictive value.  |
|                       | Alkaline elution assay           | Rat hepatocytes   | 0, 0.1 - 1.3 mM (203.2 µg/ml <sup>4</sup> )                               | Negative              | (Storer et al., 1996)                              | The experiment employed <i>d</i> -Menthol. An increase in DNA breaks was only observed at concentrations associated with cytotoxicity. The authors concluded that this was a false-positive result. The study is considered valid.   |
|                       | Sister chromatid exchange        | Chinese hamster ovary cells                                   | 5 - 50 and 0, 2 - 25 µg/ml <sup>3</sup><br>0, 16 - 167 µg/ml <sup>2</sup> | Negative <sup>1</sup> | (Ivett et al., 1989)                               | d,l-Mentol was used. The compound was tested up to toxic or nearly toxic concentration levels. The study is considered valid.  |
|                       | Sister chromatid exchange        | Human lymphocytes   | 0, 0.1, 1, 10 mM (1563 µg/ml <sup>4</sup> )                               | Negative <sup>1</sup> | (Murthy et al., 1991)                              | The study is considered valid.   |
|                       | Cytogenetic assay                | Human embryonic lung cells                                    | 0, 0.1, 1, 10 µg/ml   | Negative              | (Food and Drug Research Laboratories, Inc., 1975a) | The report does not mention exogenous metabolic activation. The study is considered valid.   |
|                       | Chromosome aberration            | Chinese hamster fibroblasts                                   | 0 and three concentrations up to 200 µg/ml                                | Negative <sup>3</sup> | (Ishidate et al., 1984)                            | The maximum concentration (cytotoxic) was selected by a preliminary test. The study is considered valid.   |
|                       | Chromosome aberration            | Chinese hamster ovary cells                                   | 0, 50 - 250 µg/ml   | Negative <sup>1</sup> | (Ivett et al., 1989)                               | d,l-Mentol was used. The compound was tested up to   |

**Table 2.2: GENOTOXICITY (*in vitro*) EFSA / FGE.09Rev3 (EFSA, 2011x) (substances in brackets are JECFA-evaluated substances)**

| Chemical Name [FL-no]     | Test system           | Test Object   | Concentration                               | Result                    | Reference                    | Comments  |
|---------------------------|-----------------------|---|---|---------------------------|------------------------------|---|
|                           | Chromosome aberration | Human lymphocytes   | 0, 0.1, 1, 10 mM (1563 µg/ml <sup>4</sup> ) | Negative <sup>1</sup>     | (Murthy et al., 1991)        | toxic or nearly toxic concentration levels. The study is considered valid.  |
|                           | Gene mutation assay   | Mouse lymphoma L5178Y TK <sup>+</sup> /cells  | 0, 12.5 - 200 µg/ml                         | Negative <sup>1</sup>     | (Myhr and Caspary, 1991)     | The study is considered valid.  |
|                           |                       |   |   |                           |                              | d,l-Menthol was used. The maximum concentration was selected by a preliminary test. The study is considered valid.  |
| (trans-Menthone [07.176]) | Ames test             | <i>S. typhimurium</i> TA97, TA98, TA100, TA1535, TA1537   | 0, 6.4 - 800 µg/plate                       | Positive <sup>1</sup>     | (Andersen and Jensen, 1984b) | Concentrations were selected based on preliminary experiments. In absence of metabolic activation, menthone was mutagenic only to strain TA1537 at 6.4 and 32 µg/ml (slightly less than 2-fold increase in mutation frequency), but not at higher (toxic) concentrations. Also in absence of metabolic activation, there was a concentration dependent increase in number of TA97 strain revertants (up to 4-fold increase at 600 µg/l). It was stated that metabolic activation did not enhance the mutagenicity of menthone. The study is considered valid. |
| Cyclopentanol [02.135]    | Modified Ames test    | <i>S. typhimurium</i> G46, TA98, TA100, TA1535, C3076, TA1537, D3052, TA1538<br><i>E. coli</i> WP2, WP2 <i>uvrA</i> | 0, 0.1 - 1000 µg/ml                         | Negative <sup>1</sup>     | (McMahon et al., 1979)       | The study was performed with agar plates containing the following concentration gradients: 0.1 - 1, 1 - 10, 10 - 100, and 100 - 1000 µg/ml. The study is considered valid, although tabulated data on cyclopentanol were not presented.   |
| (Cyclohexanone [07.148])  | Ames test             | <i>S. typhimurium</i> TA98, TA100, TA1535, TA1537   | 0, 33 - 10000 µg/plate                      | Negative <sup>1</sup>     | (Haworth et al., 1983)       | The highest level tested was the highest of either 10000 µg/plate, limit of solubility or maximal non-toxic concentration. The test was run twice. Both rat and hamster liver S9 were used. The test is considered valid.   |
|                           | Ames test             | <i>S. typhimurium</i> TA98, TA100, TA1535, TA1537   | 0, 3 µmol/plate                             | Negative <sup>1</sup>     | (Florin et al., 1980)        | A preliminary assay was performed with the four strains using only one concentration level (3 µmol/plate). This assay gave uncertain results. In addition, strains TA98 and TA100 were exposed to 0.03 - 30 µmol/plate. The validity of the study cannot be evaluated.  |
|                           | Ames test             | <i>S. typhimurium</i> TA98, TA100, TA1535, TA1537   | NR  | Positive                  | (Massoud et al., 1980)       | Only an abstract is available. No reporting with respect to metabolic activation. The substance was also tested with <i>Bacillus subtilis</i> . With this specie, toxicity was found as well as a positive response. The validity of the study cannot be evaluated because of lack of experimental information.   |
|                           | Cytogenetic assay     | Human leukocytes  | 0.1 - 10 mM                                 | Inconclusive <sup>3</sup> | (Collin, 1971)               | The study report contains little experimental detail. Gaps, but no increase in breaks, were observed without any dose response relationship. There was no information with respect to cytotoxicity or presence of a control group. Only a statement on observations from 12 cells per concentration was given, but the total number of cells studied was not specified. The study is inadequate.  |

**Table 2.2: GENOTOXICITY (*in vitro*) EFSA / FGE.09Rev3 (EFSA, 2011x) (substances in brackets are JECFA-evaluated substances)**

| Chemical Name [FL-no]                               | Test system                | Test Object  | Concentration  | Result   | Reference                 | Comments   |
|---|----------------------------|--|--|--|---------------------------|--|
|   | Chromosomal aberration     | Human lymphocytes  | 0, 0.005 - 0.1 µg/ml   | Positive                                       | (Dyshlovoi et al., 1981)  | Article is not in English. Only an abstract available in English. The validity of the study cannot be evaluated.   |
|   | Gene mutation (HPRT)       | Chinese hamster ovary cells  | 0, 7.5 µg/ml   | Negative <sup>1</sup>                          | (Aaron et al., 1985)      | Only an abstract is available with limited experimental information. The validity of the study cannot be evaluated.  |
|   | Chromosomal aberration     | Chinese hamster ovary cells  | 0, 7.5 µg/ml   | Negative <sup>1</sup>                          | (Aaron et al., 1985)      | Only an abstract is available with limited experimental information. The validity of the study cannot be evaluated.  |
|   | Sister chromatic exchange  | Chinese hamster ovary cells  | 0, 7.5 µg/ml   | Positive <sup>3</sup><br>Negative <sup>2</sup> | (Aaron et al., 1985)      | Only an abstract is available with limited experimental information. The validity of the study cannot be evaluated.  |
| Cyclohexanol [02.070]                               | Ames test                  | <i>S. typhimurium</i> TA98, TA1535, TA1537, TA1538                                 | 500 - 10000 µg/plate <sup>3</sup><br>500 - 15000 µg/plate <sup>2</sup> | Negative <sup>1</sup>                          | (Barsky, 1976)            | The highest concentrations showed cytotoxicity. The study is considered valid.   |
|   | Ames test                  | <i>S. typhimurium</i> TA98, TA100, TA1535, TA1537                                  | 0, 10 - 3333 µg/plate  | Negative <sup>1</sup>                          | (Haworth et al., 1983)    | The highest level tested was the highest of either 10000 µg/plate, limit of solubility or maximal non-toxic concentration. Both rat and hamster liver S9 were used. The test was run twice. The study is considered valid.   |
|   | Chromosomal aberration     | Human leukocytes   | 0.1 - 10 mM  | Inconclusive <sup>3</sup>                      | (Collin, 1971)            | The study report contains little experimental detail. Gaps, but no increase in breaks, were observed without any dose response relationship. There was no information with respect to cytotoxicity or presence of a control group. Only a statement on observations from 12 cells per concentration was given, but the total number of cells studied was not specified. The study is inadequate. |
| (Cyclohexyl acetate [09.027])                       | DNA damage                 | <i>B. subtilis</i> H17( <i>rec</i> <sup>+</sup> ), M45 ( <i>rec</i> <sup>-</sup> ) | 19 mg/disc   | Negative <sup>1</sup>                          | (Yoo, 1986)               |  |
| (Cyclohexyl butyrate [09.230])                      | DNA damage                 | <i>B. subtilis</i> H17( <i>rec</i> <sup>+</sup> ), M45 ( <i>rec</i> <sup>-</sup> ) | 19 mg/plate  | Negative <sup>1</sup>                          | (Oda et al., 1979)        |  |
| (Cyclopentanone [07.149])                           | Reverse mutation           | <i>S. typhimurium</i> TA98, TA100, TA1535, TA1537                                  | 2.5 - 2500 mg/plate  | Negative <sup>1</sup>                          | (Florin et al., 1980)     |  |
| (2,2,6-Trimethyl cyclohexanone [07.045])            | Reverse mutation           | <i>S. typhimurium</i> TA98, TA100, TA1535, TA1537                                  | 4.2 - 3600 mg/plate  | Negative <sup>1</sup>                          | (Florin et al., 1980)     |  |
| Methyl 3-oxo-2-pentyl-1-cyclopentylacetate [09.520] | Reverse mutation           | <i>S. typhimurium</i> TA98, TA100, TA102, TA1535, TA1537                           | 5 mg/plate   | Negative <sup>1</sup>                          | (Thompson, 2000)          | Valid study in compliance with the OECD Guideline -471.  |
|   | Reverse mutation           | <i>E. coli</i> WP2 <i>uvrA</i>   | 5 mg/plate   | Negative <sup>1</sup>                          | (Wagner and Klug, 2000)   | Valid study in compliance with the OECD Guideline -471.  |
|   | Forward mutation Test      | Mouse lymphoma cells <i>L5178y</i>   | 200 & 300 µg/L<br>300 µg/L   | Positive <sup>3</sup><br>Positive <sup>3</sup> | (Ross and Harris, 1979b)  | Pre-GLP study - not possible to assess the reliability of these studies.   |
|   | Forward mutation Test      | Mouse lymphoma cells <i>L5178y</i>   | 100 - 325 µg/L   | Negative <sup>1</sup>                          | (Cifone, 2001)            | Valid study and in compliance with OECD Guideline 476.   |
| (Carveol [02.062])                                  | Ames test (pre-incubation) | <i>S. typhimurium</i> TA98, TA100, TA1535, TA1537                                  | 560 µg/plate   | Negative                                       | (Mortelmans et al., 1986) |  |
| (Carvyl acetate [09.215])                           | Ames test (pre-incubation) | <i>S. typhimurium</i> TA98, TA100, TA1535, TA1537                                  | 333 µg/plate   | Negative                                       | (Mortelmans et al., 1986) |  |
| (L-menthyl (R,S)-3-hydroxybutyrate)                 | Reverse mutation           | <i>S. typhimurium</i> TA98, TA100, TA1535, TA1537 and TA1538                       | 78, 156, 312, 625, 1250, 2500 or 10 000 µg/plate                       | Negative <sup>ab</sup>                         | (Morimoto, 2005)          | The JECFA evaluated the racemate of L-menthyl (R,S)-3-hydroxybutyrate.   |

**Table 2.2: GENOTOXICITY (*in vitro*) EFSA / FGE.09Rev3 (EFSA, 2011x) (substances in brackets are JECFA-evaluated substances)**

| Chemical Name [FL-no] | Test system      | Test Object            | Concentration                                    | Result                  | Reference        | Comments |
|-----------------------|------------------|------------------------|--|-------------------------|------------------|----------|
|                       | Reverse mutation | <i>E. coli</i> WP2uvrA | 78, 156, 312, 625, 1250, 2500 or 10 000 µg/plate | Negative <sup>a,b</sup> | (Morimoto, 2005) |          |

NA: Not applicable.

NR: Not reported.

<sup>1</sup> With and without S9 metabolic activation.

<sup>2</sup> With S9 activation.

<sup>3</sup> Without S9 activation.

<sup>4</sup> Calculated based on molecular weight of menthol = 156.3 g/mol.

<sup>5</sup> Marked differential toxicity was seen at dose levels above 25 µmol/plate. No observations were noted at lower dose levels.



**Table 2.3: GENOTOXICITY (*in vivo*) EFSA / FGE.09Rev3 (EFSA, 2011x) (substances in brackets are JECFA-evaluated substances)**

| Chemical Name             | Test System   | Test Object   | Route                 | Dose   | Result    | Reference  | Comments  |
|---------------------------|---|---|-----------------------|--|-----------|--|---|
| (Menthol [02.015])        | Host mediated mutation assay                        | <i>S. typhimurium</i> TA1530 and G46;<br><i>S. cerevisiae</i> D3 inoculated in mice (7-9 animals/group) | Gavage                | 0, 1.45 - 5000 mg/kg bw (single dose)<br>0, 1150 mg/kg bw/day (repeated doses) | Equivocal | (Food and Drug Research Laboratories, Inc., 1975a) | Negative results, with exception of the combination <i>S. typhimurium</i> TA1530 - 5000 mg/kg bw and <i>S. cerevisiae</i> D3 - 1150 mg/kg bw/day. This study is considered valid, but the equivocal result might have low relevance since the effect was only observed at very high (lethal) dose levels. |
|                           | <i>In vivo</i> cytogenetic assay                    | Male rat bone marrow cells  | Gavage                | 0, 1.45 - 3000 mg/kg bw (single dose)<br>0, 1150 mg/kg bw/day (repeated doses) | Negative  | (Food and Drug Research Laboratories, Inc., 1975a) | Oral DL <sub>50</sub> was determined as 940 mg/kg bw. The study is considered valid but the negative result is of limited relevance, since no effect on mitotic index was observed. However, testing at higher dose levels may not have been possible, due to lethality.                                  |
|                           | <i>In vivo</i> micronucleus assay                   | B6C3F1 male mouse bone marrow cells   | Intra peritoneal      | 0, 250 - 1000 mg/kg bw/day, during 3 days                                      | Negative  | (Shelby et al., 1993)                              | d,l-Menthol was used. The study is considered valid, but the negative result is of limited relevance, since no toxicity to the bone marrow was observed. However, testing at higher dose levels was not possible, because the highest dose caused 50 % lethality.   |
|                           | <i>In vivo</i> dominant lethal assay                | Male rat fertility, spermatozoa   | Gavage                | 0, 1.45 - 3000 mg/kg bw (single dose)<br>0, 1150 mg/kg bw/day (repeated doses) | Negative  | (Food and Drug Research Laboratories, Inc., 1975a) | This study is considered valid.   |
| (trans-Menthone [07.176]) | <i>In vivo</i> SMART assay                          | <i>D. melanogaster</i> – flr3 x mwh cross   | Whole body            | 0, 1.3 µl/disk   | Positive  | (Franzios et al., 1997)                            | Somatic Mutation and Recombination Test. Only one dose level (1.29 µl/disk; slightly higher than the LD <sub>50</sub> ) was tested. A two-fold increase in mutation frequency as compared to control was observed. Menthone was not recombinogenic. The validity of this study is unclear.                |
| (Cyclohexanone [07.148])  | <i>In vivo</i> sex-linked recessive lethal mutation | <i>D. melanogaster</i>  | NR<br>3 days exposure | 0, 1 µl/ml   | Negative  | (Goncharova, 1970)                                 | Article in Russian. Only an abstract available in English. The validity of this study cannot be assessed.   |
| Cyclohexanol [02.070]     | <i>In vivo</i> sex-linked recessive lethal mutation | <i>D. melanogaster</i>  | NR<br>3 days exposure | 0, 1 µl/ml   | Negative  | (Goncharova, 1970)                                 | The validity of the study cannot be evaluated.  |
|                           | <i>In vivo</i> micronucleus test                    | NMRI mouse bone marrow  | Oral                  | 500 - 1500 mg/kg bw  | Negative  | (Gelbke, 1991)                                     | The study is considered valid. The negative result of this study is of limited relevance, since no bone marrow toxicity could be detected. Testing at higher dose levels might not have been possible due to observed general toxicity at the highest dose.   |

**Table 2.3: GENOTOXICITY (*in vivo*) EFSA / FGE.09Rev3 (EFSA, 2011x) (substances in brackets are JECFA-evaluated substances)**

| Chemical Name                                       | Test System               | Test Object     | Route            | Dose                     | Result   | Reference                   | Comments   |
|---|---------------------------|-----------------|------------------|--------------------------|----------|-----------------------------|--|
| Methyl 3-oxo-2-pentyl-1-cyclopentylacetate [09.520] | Micronucleus test         | ICR mice        | Intra peritoneal | 280, 560 & 1120 mg/kg bw | Negative | (Gudi and Krsmanovic, 1998) | Valid study in compliance with the OECD Guideline 474. |
|   | Unscheduled DNA Synthesis | Rat hepatocytes | Intra peritoneal | 333.3 & 1000 mg/kg bw    | Negative | (Durward, 2001)             | Valid study in compliance with the OECD Guideline 486. |

NR: Not reported

**Table 2.4: GENOTOXICITY (*in vitro*) from FGE.211**

| FL-no<br>JECFA-no | Chemical Name                                 | Test System            | Test Object  | Concentrations of Substance and Test Conditions             | Result   | Reference         | Comments   |
|-------------------|---|------------------------|--|---|----------|-------------------|--|
| 09.930<br>1098    | 1(7),8- <i>p</i> - Menthadien-2-yl<br>acetate | Reverse Mutation       | <i>S. typhimurium</i> TA98, TA100,<br>TA1535, TA1537 and TA102 | 1.6*, 8*, 40*, 200, 1000 and 5000 µg/plate [1,2]            | Negative | (Beevers, 2010a)  | * Concentration<br>without cytotoxicity.             |
|                   |   |                        | <i>S. typhimurium</i> TA98, TA1535<br>and TA1537               | 15.6*, 31.3*, 62.5*, 125, 250 and 500 µg/plate [2,3]        | Negative |                   |  |
|                   |   |                        | <i>S. typhimurium</i> TA100 and<br>TA102                       | 78.1*, 156.3*, 312.5, 625, 1250 and 2500 µg/plate [2,3]     | Negative |                   |  |
|                   |   |                        | <i>S. typhimurium</i> TA98 and TA100                           | 156.3*, 312.5, 625, 1250, 2500 and 5000 µg/plate [4,5]      | Negative |                   |  |
|                   |   |                        | <i>S. typhimurium</i> TA1535, TA1537<br>and TA102              | 78.1*, 156.3*, 312.5, 625, 1250 and 2500 µg/plate [4,5]     | Negative |                   |  |
|                   |   |                        | <i>S. typhimurium</i> TA100                                    | 25*, 50*, 100*, 200 and 400 µg/plate [2,3]                  | Negative |                   |  |
|                   |   |                        | <i>S. typhimurium</i> TA98                                     | 50*, 100*, 200*, 400 and 800 µg/plate [4,5]                 | Negative |                   |  |
|                   |   |                        | <i>S. typhimurium</i> TA100, TA1535,<br>TA1537 and TA102       | 25*, 50*, 100*, 200 and 400 µg/plate [4,5]                  | Negative |                   |  |
|                   |   | Micronucleus induction | Human peripheral blood<br>lymphocytes                          | 80, 90 and 110 µg/ml [3,6];<br>200, 300 and 400 µg/ml [5,6] | Negative | (Whitwell, 2010b) | 50 to 65 %<br>cytotoxicity at top<br>concentrations. |
|                   |   |                        |  | 20, 50, 80 and 100 µg/ml<br>[3,7]                           | Negative |                   |  |

[1] With and without S9 metabolic activation.

[2] Plate incorporation method.

[3] Without S9 metabolic activation.

[4] Pre-incubation method.

[5] With S9 metabolic activation.

[6] 3-hour incubation with 21-hour recovery period.

[7] 24-hour incubation with no recovery period.

**Table 2.5: GENOTOXICITY (*in vitro*) from FGE.212Rev1**

| Chemical Name [FL-no]  | Test System                 | Test Object   | Concentration  | Reported Result           | Reference  | Comments <sup>e</sup>  |
|--|-----------------------------|---|--|---------------------------|--|--|
| Tetramethyl ethylcyclohexenone (mixture of isomers [07.035]) | Reverse mutation            | <i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538 | 5 concentrations up to cytotoxicity, or max. 3600 µg/plate                   | Negative <sup>a</sup>     | (Wild et al., 1983)  | Limited validity (no TA 102 or <i>E. Coli</i> ); possibly slightly low maximal concentration tested.   |
| 3,5,5-Trimethylcyclohex-2-en-1-one [07.126]                  | Reverse mutation            | <i>S. typhimurium</i> TA97, TA98, TA100, TA1535, TA1537   | 33 - 10 000 µg/plate   | Negative <sup>a</sup>     | (Mortelmans et al., 1986)  | Valid.   |
|  | Mutation                    | <i>S. typhimurium</i> TA98, TA100, TA1535, TA1537         | 33 - 10 000 µg/plate   | Negative <sup>a</sup>     | (NTP, 1986d)   | NTP study carried out according to standard US-EPA guideline; result is considered as valid.   |
|  | Mutation                    | L5178YTk+/- mouse lymphoma cells                          | 67 - 810 µg/ml   | Negative <sup>b</sup>     | (McKee et al., 1987)   | Validity cannot be evaluated (tested with S9; abstract only with very limited information).  |
|  | Mutation                    | L5178YTk+/- mouse lymphoma cells                          | 130 - 1300 µg/ml   | Negative <sup>c</sup>     | (McKee et al., 1987)   | Validity cannot be evaluated (tested without S9; abstract only with very limited information).   |
|  | Mutation                    | L5178YTk+/- mouse lymphoma cells                          | 0.089 - 0.89 µg/ml   | Negative <sup>c</sup>     | (O'Donoghue et al., 1988)  | Valid according to current guidelines.   |
|  | Mutation                    | L5178YTk+/- mouse lymphoma cells                          | 0.13 - 1.3 µg/ml   | Negative <sup>b</sup>     | (O'Donoghue et al., 1988)  | Valid according to current guidelines.   |
|  | Mutation                    | L5178YTk+/- mouse lymphoma cells                          | 1200 µg/ml   | Positive <sup>b</sup>     | (NTP, 1986d)   | NTP study carried out according to standard US-EPA guideline; Not tested with S9. Result is considered as valid.   |
|  | Mutation                    | L5178YTk+/- mouse lymphoma cells                          | Not reported (however, up to cytotoxic concentrations) for 3 hours exposure. | Negative <sup>a</sup>     | (Honma et al., 1999a)  | Limited validity since data were presented in a summarized table format only (as a result of an international collaborative study).  |
|  | Mutation                    | L5178YTk+/- mouse lymphoma cells                          | Up to 1500 µg /ml  | Positive <sup>b</sup>     | (Honma et al., 1999b)  | Limited validity since mutation frequencies were not reported in table format. Tested only in the absence of S9. Isophorone was mutagenic after 24 hours treatments in the absence of S9. Although only graphs are plotted, it seems that increases in MF that exceeded the Global Evaluation Factor occurred at around 1250-1500 µg/ml where toxicity (by relative survival) reached 70-90 %. |
|  | Chromosomal aberration      | Chinese hamster ovary cells                               | 5 - 1600 µg/ml   | Negative <sup>a</sup>     | (Gulati et al., 1989)  | Limited validity (not clear if gaps were included in the scores).  |
|  | Chromosomal aberration      | Chinese hamster ovary cells                               | 250 - 1600 µg/ml   | Negative <sup>a</sup>     | (NTP, 1986d)   | NTP study carried out according to standard US-EPA guideline; result is considered as valid.   |
|  | Chromosomal aberration      | Chinese hamster lung fibroblasts                          | 0 - 1250 <sup>b</sup> µg/ml<br>0 - 1500 <sup>c</sup> µg/ml                   | Positive <sup>a</sup>     | (Matsuoka et al., 1996)  | Valid. Exposed to isophorone for 6 hrs with a recovery period of 18 hours.   |
|  | Chromosomal aberration      | Chinese hamster lung fibroblasts                          | 250 - 1000 mg/ml   | Negative <sup>a</sup>     | (Matsuoka et al., 1996)  | Valid. Exposed to isophorone without metabolic activation for 24 hours or 48 hours, cytotoxic at highest concentrations.   |
| Sister chromatid exchange                                    | Chinese hamster ovary cells | 5 - 1600 mg/ml  | Positive <sup>b,d</sup>  | (Gulati et al., 1989)     | Valid (pos – S9; neg + S9).  |  |
| Sister chromatid exchange                                    | Chinese hamster ovary cells | 160 - 1000 mg/ml  | Negative <sup>a</sup>  | (NTP, 1986d)              | NTP study carried out according to Standard US-EPA guideline; result is considered as valid. |  |
| Unscheduled DNA synthesis                                    | Rat hepatocytes             | 0.005 - 0.4 µl/ml   | Negative   | (O'Donoghue et al., 1988) | Valid according to current guidelines.   |  |
| Unscheduled DNA synthesis                                    | Rat hepatocytes             | 5 - 200 µl/ml   | Negative <sup>a</sup>  | (McKee et al., 1987)      | Validity cannot be evaluated (abstract only with very limited information).                  |  |

**Table 2.5: GENOTOXICITY (*in vitro*) from FGE.212Rev1**

| Chemical Name [FL-no]          | Test System                   | Test Object  | Concentration | Reported Result       | Reference                 | Comments <sup>e</sup>  |
|--------------------------------|-------------------------------|--|---------------|-----------------------|---------------------------|--|
| Carvone (isomer not specified) | Gene mutation                 | <i>S. typhimurium</i> TA1535, TA1537, TA98, TA100  | 3 µmol/plate  | Negative              | (Florin et al., 1980)     | Insufficient validity (spot test, not according to OECD guideline, methods and results insufficiently reported). Isomer (D or L) not reported. |
|                                | Rec assay                     | <i>Bacillus subtilis</i> H17 (rec+) and M45 (rec-) | 0.6 ml/disc   | Negative              | (Matsui et al., 1989)     | The test system used is considered inappropriate.  |
| <i>d</i> -Carvone [07.146]     | Gene mutation                 | <i>S. typhimurium</i> TA1535, TA98, TA100, TA1537  | 333 µg/plate  | Negative <sup>a</sup> | (NTP, 1990b)              | Valid.   |
|                                | Gene mutation (preincubation) | <i>S. typhimurium</i> TA1535, TA98, TA100, TA1537  | 560 µg/plate  | Negative              | (Mortelmans et al., 1986) | Valid.   |
|                                | Sister chromatid exchange     | Chinese hamster ovary cells                        | 502 µg/ml     | Positive <sup>a</sup> | (NTP, 1990b)              | Valid.   |
|                                | Chromosomal aberration        | Chinese hamster ovary cells                        | 400 µg/ml     | Positive <sup>a</sup> | (NTP, 1990b)              | Valid.   |

a: With and without metabolic activation.

b: Without metabolic activation.

c: With metabolic activation.

d: Cytotoxic at next highest dose tested (1600 mg/ml).

e: Validity of genotoxicity studies:

Valid.

Limited validity (e.g. if certain aspects are not in accordance with OECD guidelines or current standards and / or limited documentation).

Insufficient validity (e.g. if main aspects are not in accordance with any recognised guidelines (e.g. OECD) or current standards and/or inappropriate test system).

Validity cannot be evaluated (e.g. insufficient documentation, short abstract only, too little experimental details provided).

**Table 2.6: GENOTOXICITY (*in vivo*) from FGE.212Rev1**

| Chemical Name [FL-no]  | Test System                                  | Test Object                                      | Route  | Dose   | Result   | Reference                 | Comments <sup>a</sup>   |
|--|--|--|--------|--|----------|---------------------------|---|
| Tetramethyl ethylcyclohexenone (mixture of isomers [07.035]) | Sex-linked recessive lethal mutation         | <i>D. melanogaster</i>                           | Feed   | 10 mM  | Negative | (Wild et al., 1983)       | Limited validity (low nr of chromosomes, limited reporting)   |
|  | Micronucleus formation                       | Mouse bone marrow                                | i.p.   | 180, 307, 450 mg/kg bw   | Negative | (Wild et al., 1983)       | Limited validity. Only analysis at one time point; no PCE/NCE ratio reported  |
| 3,5,5-Trimethylcyclohex-2-en-1-one [07.126]                  | Sex-linked recessive lethal mutation         | <i>D. melanogaster</i>                           |        | 2000 <sup>b</sup> and 12 500 <sup>c</sup> ppm                                    | Negative | (Foureman et al., 1994)   | Valid, however, only limited relevance.   |
|  | Micronucleus formation                       | CD-1 mice  | i.p.   | 540 mg/kg bw (MTD)   | Negative | (McKee et al., 1987)      | Validity cannot be evaluated. Abstract only; very limited information nodata on PCE/NCE ratio.  |
|  | Micronucleus formation                       | CD-1 mice  | i.p.   | 0.54 ml/kg bw  | Negative | (O'Donoghue et al., 1988) | Limited validity. Only one dose level tested, this dose level corresponded to the LD20; sample schedule inadequate  |
|  | Chromosomal aberration                       | B6C3F1 mice                                      | i.p.   | 125, 250, 500 mg/kg bw   | Negative | NTP-Website               | Valid. Submitted by Industry in 2009. The standard protocol for <i>in vivo</i> CA is not given on the NTP website. However, based on Shelby and Witt (1995), animals should have been sampled at 17 hours and, if negative, also at 36 hours. The data on the NTP website are only for bone marrow sampled at 36 hours. It is therefore possible that a 17 hours sample was also taken, and found to be negative, but the data not posted. Fifty cells per animal were scored for CA and no increases in CA were seen. No measures of toxicity were recorded, but i.p. dosing should have guaranteed systemic exposure. |
|  | DNA binding                                  | F344 rats  | Gavage | 500 mg unlabelled isophorone / kg bw spiked with C14-isophorone (0.4 mCi/rat)    | Negative | Thier et al., 1990        | Limited validity. Submitted by Industry in 2009. No positive controls and no untreated controls used. Liver and kidney were analysed.   |
|  | DNA binding                                  | B6C3F1 mice                                      | Gavage | 500 mg unlabelled isophorone / kg bw spiked with C14-isophorone (0.08 mCi/mouse) | Negative | Thier et al., 1990        | Limited validity. Submitted by Industry in 2009. No positive controls and no untreated controls used. Liver and kidney were analysed.   |
|  | DNA binding                                  | F344 rats (10 males)                             | Gavage | 500 mg/kg bw <sup>14</sup> C-isophorone (0.1 mCi/rat)                            | Negative | Morishita et al., 1997    | Valid. Preputial glands and kidneys were analysed.  |
|  | DNA adducts ( <sup>32</sup> P-Postlabelling) | F344 rats (7 males and 7 females per dose group) | Gavage | 0 and 500 mg/kg/day for 5 days.  | Negative | Morishita et al., 1997    | Valid. Preputial glands were analysed.  |

a: Validity of genotoxicity studies:

Valid.

Limited validity (e.g. if certain aspects are not in accordance with OECD guidelines or current standards and / or limited documentation).

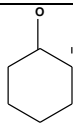
Insufficient validity (e.g. if main aspects are not in accordance with any recognised guidelines (e.g. OECD) or current standards and/or inappropriate test system).

Validity cannot be evaluated (e.g. insufficient documentation, short abstract only, too little experimental details provided).

b: Oral administration.

c: Injection.

**Table 2.7: Additional Genotoxicity Studies (*in vitro*)**

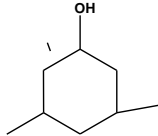
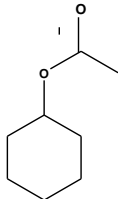
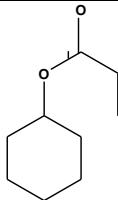
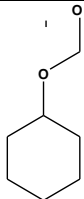
| FL-no<br>JECFA-<br>no | EU Register name<br>JECFA name | Structural formula  | End-point        | Test system   | Maximum<br>concentration | Results               | Reference   |
|-----------------------|--------------------------------|---|------------------|---|--------------------------|-----------------------|-------------|
| 07.148<br>1100        | Cyclohexanone                  |  | Reverse mutation | <i>S. typhimurium</i> TA98,<br>TA100, TA1535,<br>TA1537 | 33–3333 µg/plate         | Negative <sup>a</sup> | (NTP, 2007) |
|                       |                                |   | Mutation         | Mouse lymphoma<br>L5178Y Tk <sup>+/+</sup> cells        | 312.5–5000 µg/ml         | Negative              | (NTP, 2007) |

<sup>a</sup> With and without metabolic activation.

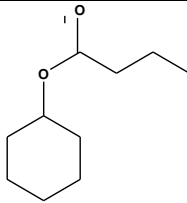
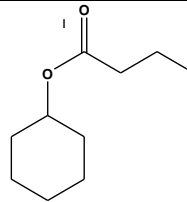
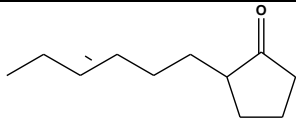
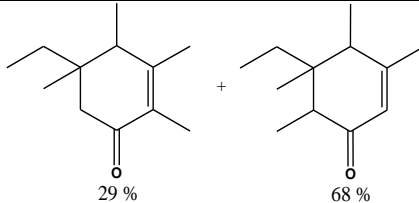
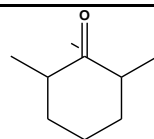


**TABLE 3: SUMMARY OF SAFETY EVALUATIONS**

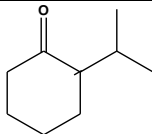
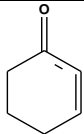
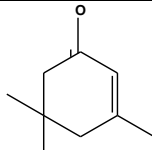
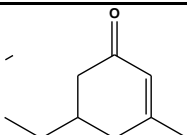
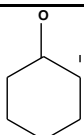
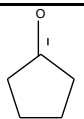
**Table 3.1: Summary of Safety Evaluation of Alicyclic Ketones, Secondary Alcohols and Related Esters (JECFA, 2003a)**

| FL-no<br>JECFA-no | EU Register name               | Structural formula  | EU MSDI 1)<br>US MSDI<br>( $\mu\text{g/capita/day}$ ) | Class 2)<br>Evaluation procedure<br>path 3) | Outcome on the<br>named compound<br>[4) or 5)] | EFSA conclusion on the<br>named compound<br>(Procedure steps, intake<br>estimates, NOAEL,<br>genotoxicity) | EFSA conclusion on the<br>material of commerce                                 |
|-------------------|--------------------------------|---|---|---|--|--|--|
| 02.209<br>1099    | 3,3,5-Trimethylcyclohexan-1-ol |    | 0.12<br>0.1   | Class I<br>A3: Intake below threshold       | 4)   | No safety concern at the estimated level of intake based on the MSDI approach.                             | No safety concern at the estimated level of intake based on the MSDI approach. |
| 09.027<br>1093    | Cyclohexyl acetate             |    | 12<br>10  | Class I<br>A3: Intake below threshold       | 4)   | No safety concern at the estimated level of intake based on the MSDI approach.                             | No safety concern at the estimated level of intake based on the MSDI approach. |
| 09.140<br>1097    | Cyclohexyl propionate          |   | 0.012<br>0.05   | Class I<br>A3: Intake below threshold       | 4)   | No safety concern at the estimated level of intake based on the MSDI approach.                             | No safety concern at the estimated level of intake based on the MSDI approach. |
| 09.160<br>1095    | Cyclohexyl formate             |  | 0.012<br>0.2  | Class I<br>A3: Intake below threshold       | 4)   | No safety concern at the estimated level of intake based on the MSDI approach.                             | No safety concern at the estimated level of intake based on the MSDI approach. |

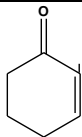
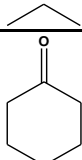
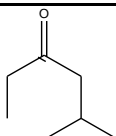
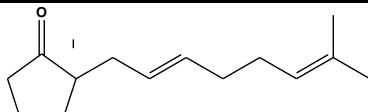
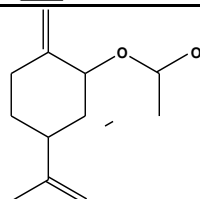
**Table 3.1: Summary of Safety Evaluation of Alicyclic Ketones, Secondary Alcohols and Related Esters (JECFA, 2003a)**

| FL-no<br>JECFA-no | EU Register name                                    | Structural formula  | EU MSDI 1)<br>US MSDI<br>( $\mu\text{g/capita/day}$ ) | Class 2)<br>Evaluation procedure<br>path 3) | Outcome on the<br>named compound<br>[4) or 5)] | EFSA conclusion on the<br>named compound<br>(Procedure steps, intake<br>estimates, NOAEL,<br>genotoxicity)  | EFSA conclusion on the<br>material of commerce                                 |
|-------------------|---|---|---|---|--|---|--|
| 09.230<br>1094    | Cyclohexyl butyrate                                 |    | 0.89<br>0.1   | Class I<br>A3: Intake below threshold       | 4)   | No safety concern at the estimated level of intake based on the MSDI approach.  | No safety concern at the estimated level of intake based on the MSDI approach. |
| 09.464<br>1096    | Cyclohexyl isovalerate                              |    | 0.28<br>0.05  | Class I<br>A3: Intake below threshold       | 4)   | No safety concern at the estimated level of intake based on the MSDI approach.  | No safety concern at the estimated level of intake based on the MSDI approach. |
| 07.034<br>1106    | 2-Hexyldenecyclopentan-1-one                        |    | 0.24<br>0.01  | Class II<br>A3: Intake below threshold      | 4)   | Evaluated in FGE.211, genotoxicity concern could be ruled out.<br>No safety concern at the estimated level of intake based on the MSDI approach.  | No safety concern at the estimated level of intake based on the MSDI approach. |
| 07.035<br>1111    | Tetramethyl ethylcyclohexenone (mixture of isomers) |   | 7.8<br>0.2  | Class II<br>A3: Intake below threshold      | 4)   | Evaluated in FGE.212Rev1, genotoxic concern could be ruled out.<br>No safety concern at the estimated level of intake based on the MSDI approach. | No safety concern at the estimated level of intake based on the MSDI approach. |
| 07.045<br>1108    | 2,2,6-Trimethylcyclohexanone                        |  | 2.1<br>0.04   | Class II<br>A3: Intake below threshold      | 4)   | No safety concern at the estimated level of intake based on the MSDI approach.  | No safety concern at the estimated level of intake based on the MSDI approach. |

**Table 3.1: Summary of Safety Evaluation of Alicyclic Ketones, Secondary Alcohols and Related Esters (JECFA, 2003a)**

| FL-no<br>JECFA-no | EU Register name                     | Structural formula  | EU MSDI 1)<br>US MSDI<br>( $\mu\text{g/capita/day}$ ) | Class 2)<br>Evaluation procedure<br>path 3) | Outcome on the<br>named compound<br>[4) or 5)] | EFSA conclusion on the<br>named compound<br>(Procedure steps, intake<br>estimates, NOAEL,<br>genotoxicity)  | EFSA conclusion on the<br>material of commerce   |
|-------------------|--------------------------------------|---|---|---|--|---|--|
| 07.095<br>1109    | 2-(sec-Butyl)cyclohexanone           |    | 5.1<br>ND   | Class II<br>A3: Intake below threshold      | 4)   | No safety concern at the estimated level of intake based on the MSDI approach.  | According to JECFA: Min. assay value is "94%" and secondary components "2-Isobutyl cyclohexanone" No safety concern at the estimated level of intake based on the MSDI approach. |
| 07.098<br>1107    | 3-Methylcyclohex-2-en-1-one          |    | 0.012<br>0.1  | Class II<br>A3: Intake below threshold      | 4)   | Evaluated in FGE.212Rev1, genotoxic concern could be ruled out.<br>No safety concern at the estimated level of intake based on the MSDI approach. | No safety concern at the estimated level of intake based on the MSDI approach.   |
| 07.126<br>1112    | 3,5,5-Trimethylcyclohex-2-en-1-one   |    | 4.6<br>0.1  | Class II<br>A3: Intake below threshold      | 4)   | Evaluated in FGE.212Rev1, genotoxic concern could be ruled out.<br>No safety concern at the estimated level of intake based on the MSDI approach. | No safety concern at the estimated level of intake based on the MSDI approach.   |
| 07.129<br>1113    | 3-Methyl-5-propylcyclohex-2-en-1-one |   | 0.097<br>4.1  | Class II<br>A3: Intake below threshold      | 4)   | Evaluated in FGE.212Rev1, genotoxic concern could be ruled out.<br>No safety concern at the estimated level of intake based on the MSDI approach. | No safety concern at the estimated level of intake based on the MSDI approach.   |
| 07.148<br>1100    | Cyclohexanone                        |  | 0.12<br>0.1   | Class II<br>A3: Intake below threshold      | 4)   | No safety concern at the estimated level of intake based on the MSDI approach.  | No safety concern at the estimated level of intake based on the MSDI approach.   |
| 07.149<br>1101    | Cyclopentanone                       |  | 0.018<br>0.02   | Class II<br>A3: Intake below threshold      | 4)   | No safety concern at the estimated level of intake based on the MSDI approach.  | No safety concern at the estimated level of intake based on the MSDI approach.   |


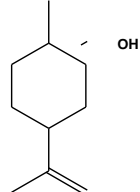
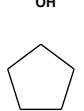
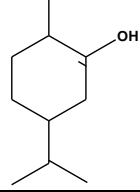
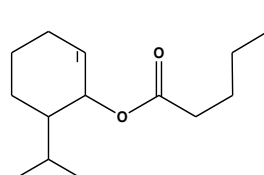
**Table 3.1: Summary of Safety Evaluation of Alicyclic Ketones, Secondary Alcohols and Related Esters (JECFA, 2003a)**

| FL-no<br>JECFA-no | EU Register name  | Structural formula  | EU MSDI 1)<br>US MSDI<br>( $\mu\text{g/capita/day}$ ) | Class 2)<br>Evaluation procedure<br>path 3) | Outcome on the<br>named compound<br>[4) or 5)] | EFSA conclusion on the<br>named compound<br>(Procedure steps, intake<br>estimates, NOEL,<br>genotoxicity)   | EFSA conclusion on the<br>material of commerce                                 |
|-------------------|---|---|---|---|--|---|--|
| 07.172<br>1110    | 4-Isopropylcyclohex-2-en-1-one                                    |    | 0.0012<br>0.001                                       | Class II<br>A3: Intake below threshold      | 4)   | Evaluated in FGE.212Rev1, genotoxic concern could be ruled out.<br>No safety concern at the estimated level of intake based on the MSDI approach. | No safety concern at the estimated level of intake based on the MSDI approach. |
| 07.179<br>1102    | 2-Methylcyclohexanone   |    | 0.12<br>0.1   | Class II<br>A3: Intake below threshold      | 4)   | No safety concern at the estimated level of intake based on the MSDI approach.  | No safety concern at the estimated level of intake based on the MSDI approach. |
| 07.180<br>1103    | 3-Methylcyclohexanone   |    | 0.12<br>0.1   | Class II<br>A3: Intake below threshold      | 4)   | No safety concern at the estimated level of intake based on the MSDI approach.  | No safety concern at the estimated level of intake based on the MSDI approach. |
| 07.257<br>1117    | 2-(3,7-Dimethyl-2,6-octadienyl) cyclopentanone                    |    | 3.0<br>6.6  | Class II<br>A3: Intake below threshold      | 4)   | No safety concern at the estimated level of intake based on the MSDI approach.  | No safety concern at the estimated level of intake based on the MSDI approach. |
| 09.930<br>1098    | 1(7),8-p-Menthadien-2-yl acetate (mixture of (E) and (Z) isomers) |  | 0.61<br>0.6   | Class II<br>A3: Intake below threshold      | 4)   | Evaluated in FGE.211, genotoxicity concern could be ruled out.<br>No safety concern at the estimated level of intake based on the MSDI approach.  | No safety concern at the estimated level of intake based on the MSDI approach. |

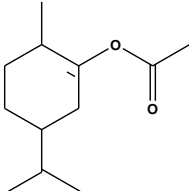
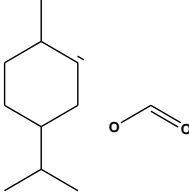
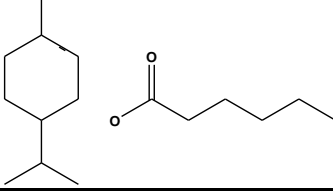
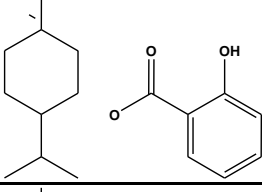
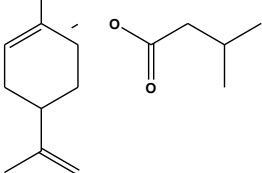
- 1) EU MSDI: Amount added to food as flavour in ( $\text{kg / year}$ )  $\times 10^9$  / ( $0.1 \times$  population in Europe (=  $375 \times 10^6$ )  $\times 0.6 \times 365$ ) =  $\mu\text{g/capita/day}$ .
- 2) Thresholds of concern: Class I =  $1800 \mu\text{g/person/day}$ , Class II =  $540 \mu\text{g/person/day}$ , Class III =  $90 \mu\text{g/person/day}$ .
- 3) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.
- 4) No safety concern based on intake calculated by the MSDI approach of the named compound.
- 5) Data must be available on the substance or closely related substances to perform a safety evaluation.

ND: not determined

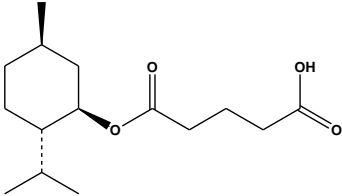
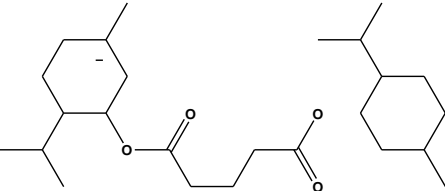
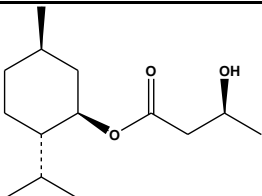
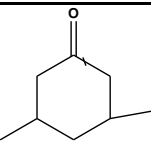
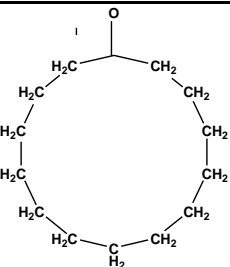
**Table 3.2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach) (EFSA / FGE.09Rev3)**

| FL-no          | EU Register name   | Structural formula  | MSDI 1)<br>(µg/capita/day) | Class 2)<br>Evaluation procedure path<br>3) | Outcome on the named<br>compound<br>[ 4) or 5] | Outcome on the<br>material of<br>commerce [6), 7),<br>or 8)] | Evaluation remarks |
|----------------|--------------------|---|----------------------------|---|--|--|--------------------|
| 02.070         | Cyclohexanol       |    | 3.7                        | Class I<br>A3: Intake below threshold       | 4)   | 6)   |                    |
| 02.075         | neo-Dihydrocarveol |    | 2.4                        | Class I<br>A3: Intake below threshold       | 4)   | 6)   |                    |
| 02.135         | Cyclopentanol      |    | 0.012                      | Class I<br>A3: Intake below threshold       | 4)   | 6)   |                    |
| 02.167         | Isodihydrocarveol  |   | 2.4                        | Class I<br>A3: Intake below threshold       | 4)   | 6)   |                    |
| 09.154<br>1852 | Menthyl valerate   |  | 1.0                        | Class I<br>A3: Intake below threshold       | 4)   | 6)   |                    |

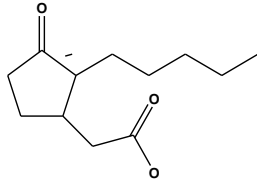
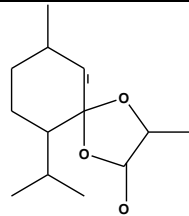
**Table 3.2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach) (EFSA / FGE.09Rev3)**

| FL-no  | EU Register name          | Structural formula  | MSDI 1)<br>(µg/capita/day) | Class 2)<br>Evaluation procedure path<br>3) | Outcome on the named<br>compound<br>[ 4) or 5] | Outcome on the<br>material of<br>commerce [6), 7),<br>or 8)] | Evaluation remarks |
|--------|---------------------------|---|----------------------------|---|--|--|--------------------|
| 09.355 | neo-Dihydrocarvyl acetate |    | 0.012                      | Class I<br>A3: Intake below threshold       | 4)   | 6)   |                    |
| 09.618 | Menthyl formate           |    | 0.73                       | Class I<br>A3: Intake below threshold       | 4)   | 6)   |                    |
| 09.619 | Menthyl hexanoate         |    | 0.37                       | Class I<br>A3: Intake below threshold       | 4)   | 6)   |                    |
| 09.621 | Menthyl salicylate        |   | 0.012                      | Class I<br>A3: Intake below threshold       | 4)   | 6)   |                    |
| 09.870 | Carvyl-3-methylbutyrate   |  | 0.0012                     | Class I<br>A3: Intake below threshold       | 4)   | 6)   | a)                 |

**Table 3.2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach) (EFSA / FGE.09Rev3)**

| FL-no  | EU Register name                | Structural formula  | MSDI 1)<br>(µg/capita/day) | Class 2)<br>Evaluation procedure path 3)                         | Outcome on the named compound<br>[ 4) or 5] | Outcome on the material of commerce [6), 7), or 8)] | Evaluation remarks |
|--------|---------------------------------|---|----------------------------|--|---|---|--------------------|
| 09.929 | L-Monomenthyl glutarate         |    | 110                        | Class I<br>A3: Intake below threshold                            | 4)  | 6)  |                    |
| 09.935 | Dimenthyl glutarate             |   | 30                         | Class I<br>A3: Intake below threshold                            | 4)  | 6)  |                    |
| 09.949 | L-Menthyl (S)-3-hydroxybutyrate |    | 37                         | Class I<br>A3: Intake below threshold                            | 4)  | 6)  |                    |
| 07.203 | 3,3,5-Trimethylcyclohexan-1-one |   | 0.0085                     | Class II<br>A3: Intake below threshold                           | 4)  | 6)  |                    |
| 07.207 | Cyclotetradecanone              |  | 0.061                      | Class II<br>B3: Intake below threshold,<br>B4: No adequate NOAEL | Additional data required                    |   |                    |

**Table 3.2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach) (EFSA / FGE.09Rev3)**

| FL-no          | EU Register name  | Structural formula  | MSDI 1)<br>(µg/capita/day) | Class 2)<br>Evaluation procedure path 3)  | Outcome on the named compound<br>[ 4) or 5] | Outcome on the material of commerce [6), 7), or 8)] | Evaluation remarks |
|----------------|---|---|----------------------------|---|---|---|--------------------|
| 09.520         | Methyl 3-oxo-2-pentyl-1-cyclopentylacetate              |  | 770                        | Class II<br>A3: Intake above threshold,<br>A4: Not endogenous, A5:<br>Adequate NOAEL exists | 4)  | 6)  |                    |
| 06.136<br>1859 | 6-Isopropyl-3,9-dimethyl-1,4-dioxyspiro[4.5]decan-2-one |  | 12                         | Class III<br>A3: Intake below threshold   | 4)  | 6)  |                    |

- 1) EU MSDI: Amount added to food as flavour in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day.
- 2) Thresholds of concern: Class I = 1800 µg/person/day, Class II = 540 µg/person/day, Class III = 90 µg/person/day.
- 3) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.
- 4) No safety concern based on intake calculated by the MSDI approach of the named compound.
- 5) Data must be available on the substance or closely related substances to perform a safety evaluation.
- 6) No safety concern at estimated level of intake of the material of commerce meeting the specification of Table 1 (based on intake calculated by the MSDI approach).
- 7) Tentatively regarded as presenting no safety concern (based on intake calculated by the MSDI approach) pending further information on the purity of the material of commerce and/or information on stereoisomerism.
- 8) No conclusion can be drawn due to lack of information on the purity of the material of commerce.
- a) Evaluated in FGE.212, genotoxic concern could be ruled out.



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## ABBREVIATIONS

|             |   |
|-------------|---|
| ATP         | Adenosine Tri-Phosphate   |
| BW          | Body weight   |
| CA          | Chromosomal Aberration  |
| CAS         | Chemical Abstract Service   |
| CEF         | Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids |
| CHO         | Chinese hamster ovary (cells)   |
| CoE         | Council of Europe   |
| DNA         | Deoxyribonucleic acid   |
| EFSA        | The European Food Safety Authority  |
| EPA         | United States Environmental Protection Agency                             |
| EU          | European Union  |
| FAO         | Food and Agriculture Organization of the United Nations                   |
| FEMA        | Flavor and Extract Manufacturers Association                              |
| FGE         | Flavouring Group Evaluation   |
| FLAVIS (FL) | Flavour Information System (database)                                     |
| GEF         | Global Evaluation Factor  |
| GLP         | Good laboratory practise  |
| ID          | Identity  |
| Ip          | Intraperitoneal   |
| IR          | Infrared spectroscopy   |
| JECFA       | The Joint FAO/WHO Expert Committee on Food Additives                      |
| MF          | Mutation Frequency  |
| MLA         | Mouse Lymphoma Assay  |
| MNBN        | Micronucleated binucleate cells   |
| MSDI        | Maximised Survey-derived Daily Intake                                     |
| mTAMDI      | Modified Theoretical Added Maximum Daily Intake                           |
| NCE         | Normochromatic erythrocyte  |
| No          | Number  |



|       |  |
|-------|--|
| NOAEL | No observed adverse effect level                       |
| NTP   | National Toxicology Program                            |
| OECD  | Organisation for Economic Co-operation and Development |
| PCE   | Polychromatic erythrocyte                              |
| SCE   | Sister chromatic exchange                              |
| SCF   | Scientific Committee on Food                           |
| SLRL  | Sex Linked Recessive Lethal Mutations test             |
| SMART | Somatic Mutation And Recombination Test                |
| UDS   | Unscheduled DNA Synthesis                              |
| WHO   | World Health Organisation                              |